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OF BIOLOGICAL CELLS

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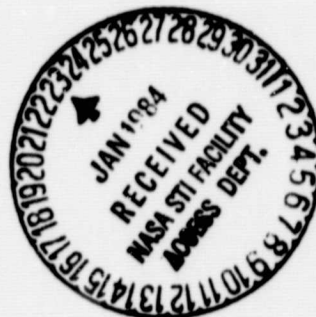
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## ABSTRACT

Detailed physicochemical studies of dextran/poly(ethylene glycol) (PEG) two phase systems have been carried out to characterize and provide understanding of the properties of the systems which determine cell partition and the electrophoretic behavior of phase drops responsible for electric field-driven phase separation. Since we have shown that the mobilities are strong functions of the salt concentration and salt partition in the phase systems, a detailed study of the electrostatic and electrokinetic potentials developed in these systems was carried out. The salt partition was examined both in phase systems and with pure polymer solutions via equilibrium dialysis and mechanism of sulfate, chloride and phosphate partition shown to be exclusion by PEG rather than binding by dextran. Salt partition was shown to have a strong effect on the polymer compositions of the phases as well, an effect which produces large changes in the interfacial tension between them. These effects were characterized and the interfacial tension shown to obey a power law with respect to its dependence on the length of the tie line describing the system composition on a phase diagram. The electrostatic potential differences measured via salt bridges were shown to obey thermodynamic predictions. The electrophoretic mobilities measured were utilized to provide a partial test of Levine's incomplete theory of phase drop electrophoresis. The data were consistent with Levine's expression over a limited range of the variables tested.

## INTRODUCTION

The body of this report consists of the four papers in press or submitted which contain the results of the effort expended on this contract. Appended also are abstracts of presentations made of this work, where they were prepared. These publications, abstracts and presentations are listed below.

## PUBLICATIONS

1. Brooks, D.E. and Bamberger, S. Studies on aqueous two phase polymer systems useful for partitioning of biological materials. In: Materials Processing in the Reduced Gravity Environment of Space (G.E. Rindone, ed.), Elsevier Science, New York, 1982, pp. 233-240.
2. Bamberger, S., Seaman, G.V.F., Brown, J.A. and Brooks, D.E. The partition of sodium phosphate and sodium chloride in aqueous dextran poly(ethylene glycol) two phase systems. J. Colloid Interface Sci. (In press).
3. Bamberger, S., Seaman, G.V.F., Sharp, K.A. and Brooks, D.E. The effects of salts on the interfacial tension of aqueous dextran poly(ethylene glycol) phase systems. J. Colloid Interface Sci. (In press).
4. Brooks, D.E., Sharp, K.A., Bamberger, S., Tamblyn, C.H., Seaman, G.V.F. and Walter, H. Electrostatic and electrokinetic potentials in two polymer aqueous phase systems. J. Colloid Interface Sci. (Submitted for publication).

## ABSTRACTS AND PRESENTATIONS

1. Bamberger, S., Seaman, G.V.F. and Brooks, D.E. Studies on the interfacial tension of dextran/PEG systems. Second International Conference on Partitioning, Sheffield, England, April 5-8, 1981.
2. Brooks, D.E. and Bamberger, S. Studies on aqueous two phase polymer systems useful for partitioning of biological materials. Symposium on Materials

Processing Research in the Reduced Gravity Environment of Space. Annual Meeting of the Materials Research Society, Boston, MA, November 16-18, 1981.

3. Bamberger, S., Seaman, G.V.F. and Brooks, D.E. Electrostatic potentials in two polymer aqueous phase systems. 57th Colloid and Surface Science Symposium, Toronto, Ontario, June 12-15, 1983.
4. Sharp, K.A., Evans, E.A. and Brooks, D.E. Cell partitioning in aqueous polymer two phase systems and cell surface free energies related through three phase contact angles. 57th Colloid and Surface Science Symposium, Toronto, Ontario, June 12-15, 1983.
5. Bamberger, S., Seaman, G.V.F., Sharp, K.A. and Brooks, D.E. Electrical potentials across the interphase in two polymer aqueous phase systems. Third International Conference on Partitioning in Two Polymer Systems, Vancouver, B.C., July 3-8, 1983.
6. Brooks, D., Bamberger, S., Sharp, K.A. and Van Alstine, J. Gravitational limitations on cell partition separations. Third International Conference on Partitioning in Two Polymer Systems, Vancouver, B.C., July 3-8, 1983.
7. Sharp, K., Evans, E.A. and Brooks, D.E. Cell partition and surface free energies related through contact angles in two phase polymer systems. Third International Conference on Partitioning in Two Polymer Systems, Vancouver, B.C., July 3-8, 1983.

## STUDIES ON AQUEOUS TWO PHASE POLYMER SYSTEMS USEFUL FOR PARTITIONING OF BIOLOGICAL MATERIALS

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### ABSTRACT

The two phase systems that result when aqueous solutions of dextran and poly(ethylene glycol) (PEG) are mixed above a critical concentration of a few percent provide a useful medium for the separation of biological cell subpopulations via partition between the top, PEG-rich phase and the liquid-liquid phase boundary. Interfacial tensions of such systems have been measured by the rotating drop technique and found to range between  $10^{-1}$  and  $10^2$   $\mu\text{N m}^{-1}$ . The tension was found to depend on the length of the tie line describing the system on a phase diagram, via a power law relationship which differed depending on the concentration of Na phosphate buffer present. The electrokinetic properties of drops of one phase suspended in the other were studied for a variety of systems. It was found that the droplet electrophoretic mobility increased monotonically with phosphate concentration and drop diameter but exhibited the opposite sign from that anticipated from phosphate partition measurements. It was possible to take advantage of these electrokinetic properties and dramatically enhance the speed of phase separation through application of relatively small electric fields.

### INTRODUCTION

The two phase systems which form when dextran (poly( $\alpha$ -1,6 glucose)) and poly(ethylene glycol) (PEG) are mixed to form aqueous solutions which are greater than a few percent in each, when appropriately buffered, have proven to be valuable as partition media for macromolecules, sub-cellular organelles and whole biological cells [1-3]. When cells or microscopic particles are introduced to such systems, the systems shaken to emulsify the phases, and allowed to re-settle, it is frequently found that the cells are distributed between the top, PEG-rich phase and the interface between the bulk phases, the dextran-rich phase being empty of cells. The interface therefore acts as a third phase with respect to the distribution of particulates in the systems.

The strength of this procedure as an analytical or preparative process derives from the fact that the partition coefficient (percentage of cells found in the top phase) is determined, ideally, by cell membrane properties which are under direct genetic control and which can vary in ways relevant to cell activity and function. While for macromolecules the partition coefficient is determined purely thermodynamically, for particulates such as cells which do not diffuse to any great degree because of their size (1-50  $\mu\text{m}$  diameter) the determining mechanisms are not well understood.

Partitioning works well for relatively small cells. An inherent limitation appears for cells which sediment significantly during the time required for the phases to settle, however, since such cells will sediment into the interface, or to the bottom of the tube, before phase separation is complete. By working in a low gravity environment cell sedimentation would be eliminated, but an additional driving force for phase isolation would have to be introduced. We have been investigating the possibility of using an externally applied electric field as a means to collect and isolate the phases. In addition, since the initial stage of the separation process should be partially determined by the interfacial tension of the liquid-liquid interface between the phases, we are studying this parameter as a function of system variables. Our results to date are summarized below.

#### MATERIALS AND METHODS

Phase systems. These were made up by weight from concentrated stock salt solutions and 25%-35% w/w stock solutions of dextran T40 (Pharmacia Fine Chemicals; lot 7974;  $M_v = 41,500$ ;  $M_n = 26,800$ ) and PEG 6000 (Union Carbide; lot R-529-9104;  $M_v = 6000$ )<sup>n</sup> in twice glass distilled water. Phase systems were allowed to settle at the desired temperature until neither phase showed visible turbidity, then each phase isolated. Polymer concentrations of the stocks and separated phases were measured polarimetrically (dextran) or refractometrically (PEG) as described [1]. Phosphate concentrations were measured by the stannous chloride method [4] following removal of interfering organics with  $HNO_3/H_2SO_4$  [4]. Phase densities were determined pycnometrically.

Interfacial tension. The rotating drop method [5] was used, employing an apparatus constructed locally, designed after the descriptions of Vonnegut [5], Princen et al. [6] and Ryden and Albertsson [7]. The equilibrium lengths of drops of known volume of the PEG-rich phase suspended in the dextran-rich phase were determined with a travelling microscope at measured rotation rates between 300 and 2500 rpm. The interfacial tensions were calculated from the above measurements and the phase densities using the formula and tables given by Princen et al. [6].

Phase droplet electrophoretic mobility. Small volumes of one of the isolated phases were injected into large volumes of the other (2-4  $\mu$ l per ml), the dispersion mixed gently and introduced into the cylindrical chamber of a microelectrophoresis apparatus (Rank Bros.) equipped with a filar eyepiece micrometer (American Optical Co.). Droplet diameters were measured to  $\pm 0.4 \mu$ m using the micrometer, with the field off, before the mobility of each was measured. Mobilities were determined by recording the time necessary for a drop to move a known distance, calculating the velocity, and dividing by the electric field strength applied [8].

Electric field-driven phase separation (FDPS). FDPS was carried out in an apparatus described in detail in the NASA patent documents covering the process [9]. Briefly, the separation cell, made from poly(methyl methacrylate) consists of a sample chamber approximately 0.5 cm W x 5 cm L x 0.2 cm H, its upper and lower boundaries consisting of membranes (Amicon UM2) which separate it from the upper and lower electrode chambers. The design is such that the Rayleigh Number is always less than 100 in the separation chamber under operating conditions, implying that no unstable convection will occur [10]. The electric field is applied vertically via bright Pt wire electrodes. Buffer is continually passed through both electrode chambers to remove the gases generated and to remove heat at the separation

chamber boundaries. A mixed phase system is introduced into the separation chamber via a loading port. The time course of phase separation is followed turbidimetrically through measurement of the intensity of the beam of a ruby laser which traverses the width of the chamber roughly 0.1 cm from the bottom membrane and impinges on a solid state detector. The output from the detector is amplified and displayed on a chart recorder. Introduction of a mixed, turbid phase system scatters most of the beam away from the detector, producing a minimum signal. As phase separation occurs the system clears optically and the transmitted intensity increases correspondingly. It is also possible to isolate the top and bottom halves of the sample chamber since the unit is split in a plane parallel with, and half way between, the upper and lower membranes. The top half can then be slid off the bottom half laterally onto a flat plate, isolating the liquid in the top half of the chamber and leaving the material in the bottom half similarly trapped.

## RESULTS AND DISCUSSION

Figure 1 shows the phase diagram for dextran 40, PEG 6000 mixtures at 22°C in the presence of different salts and salt concentrations. It is clear that whereas 0.15M NaCl had no measurable effect on phase separation, Na phosphate buffers (equimolar  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$  solutions mixed to produce the desired pH) at pH 7.5 progressively depressed the binodial and critical point as the phosphate concentration was increased. This implies that the polymer concentration differences between two phases formed from systems containing the same total amount of dextran and PEG will be greater the greater the phosphate concentration. A parallel effect has been found in the partition of the phosphate salts in these systems (measured as total P), the phosphate concentration in the dextran-rich bottom phase being greater than that in the top phase and the effect increasing with increasing salt concentration (Table 1). This unequal salt partition produces a Donnan

TABLE 1

Total phosphorous partition in a phase system containing 10.7% w/w dextran 40, 4.3% PEG 6000, pH 7.5

Phosphate conc'n (M)	Tie line length (% w/w)	Phosphate partition coefficient <sup>a)</sup>
0.22	19.0	0.622
0.11	18.4	0.665
0.01	16.3	0.754

<sup>a)</sup> Phosphate partition coefficient = molar concentration ratio, top/bottom

potential between the phases [11] which is believed to influence the partition behavior of charged particles and cells.

The interfacial tensions measured at 22°C for the systems analyzed in Figure 1 are given in Figure 2. The parameter which characterizes the phase system in this plot is the tie line length (TLL), the distance on the phase diagram between the two points on the binodial which give the composition of the separated phases. It is seen that the tensions are in the range  $10^{-1}$



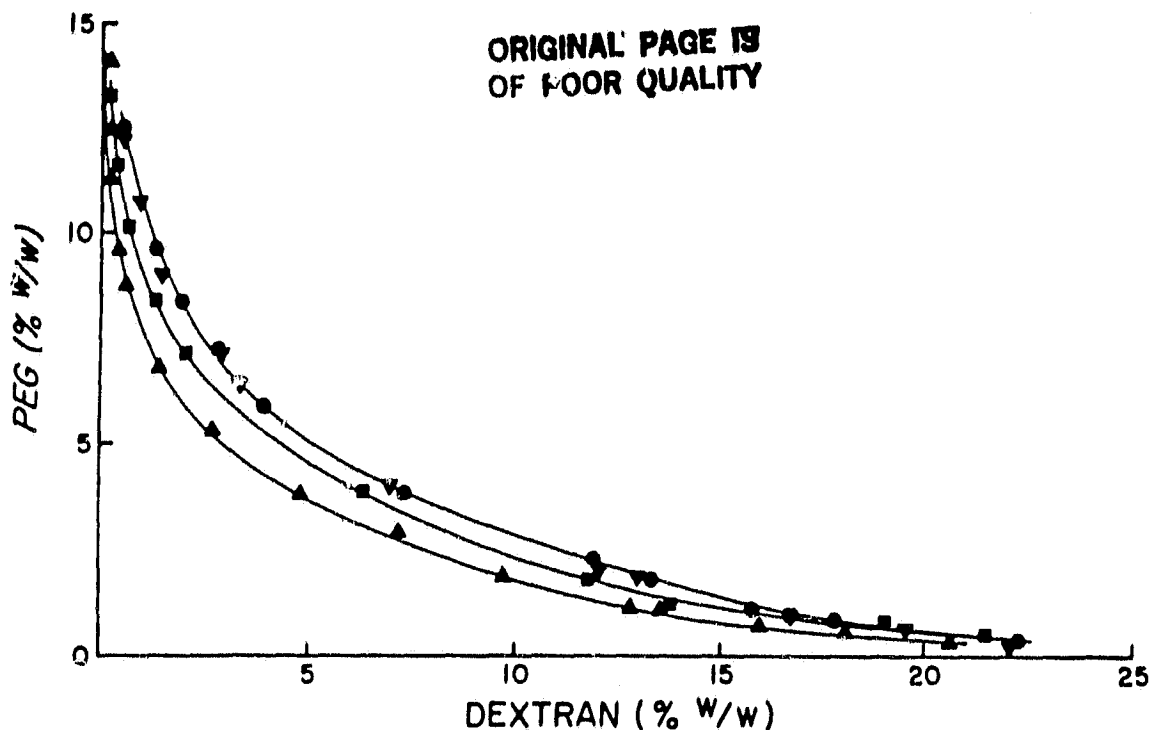


Fig. 1. The dextran T40, PEG 6000 phase diagram at 22°C. Systems contained: (●) no salt; (▼) 0.15 M NaCl; (■) 0.11 M Na phosphate buffer, pH 7.5; (▲) 0.22 M Na phosphate buffer, pH 7.5.

to  $10^2 \mu\text{N m}^{-1}$  ( $10^{-4}$ – $10^{-1}$  dyne  $\text{cm}^{-1}$ ). The log-log plots are strictly linear over the range of compositions examined, the slopes being 3.64 (no salt), 3.77 (0.11 M phosphate) and 3.52 (0.22 M phosphate). Hence the tension varies as TLL raised to these powers.

The conclusion that the interfacial tension shows a power law dependence on TLL is at variance with that of Ryden and Albertsson [7] who found, working over a more limited composition range, that the log of the tension depended directly on TLL. Such a relationship clearly could not apply at  $\text{TLL} = 0$ , however. In fact, Ryden and Albertsson's data agrees quite well with ours where the two sets overlap.

The other noteworthy feature of Figure 2 is that the systems containing phosphate exhibit unique behavior for each concentration. The effect of phosphate is not just to drive the system further from the critical point and increase the TLL. Systems with the same tie line length have higher tensions the higher the phosphate concentration. This suggests that the value of the interfacial tension reflects to some extent the magnitude of the salt concentration gradient across the phase boundary as well as those of the two polymers in the systems.

The results of electrokinetic studies on droplets of one phase suspended in the other for various systems are given in Figures 3 to 5. Figure 3 shows that drops of the top, PEG-rich phase exhibited a negative electrophoretic mobility and drops of the bottom, dextran-rich phase a positive in experiments in which one or the other was the disperse phase. The magnitude of the mobility increased with increasing phosphate concentration (in the presence of sufficient supporting NaCl to maintain the ionic strength approximately constant). Remarkably, the sign of the mobility is opposite to that expected from the phosphate partition and the Donnan potential,

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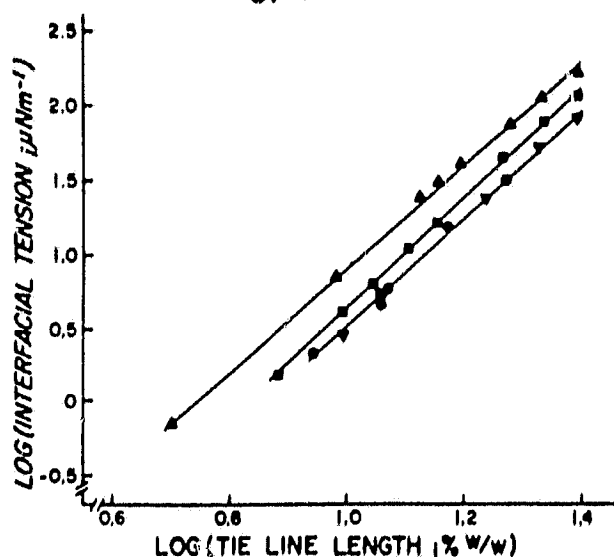


Fig. 2. Log-log plot of interfacial tension as a function of tie line length;  $T = 22^\circ\text{C}$ ; symbols as for Fig. 1.

dextran-rich drops exhibiting a positive mobility in spite of containing a higher phosphate concentration than the surrounding PEG-rich phase.

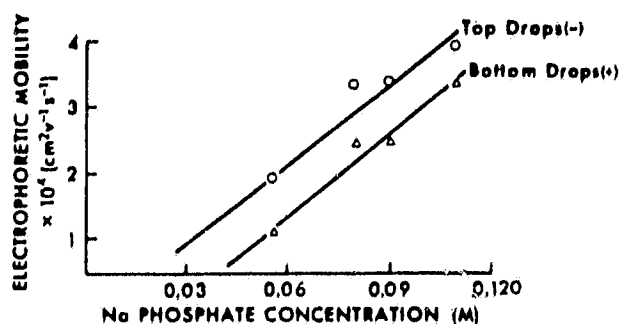


Fig. 3. Electrophoretic mobility of droplets as a function of Na phosphate buffer concentration, pH 7.5, in a phase system containing 7.5% dextran T40, 4.5% PEG 6000 plus, for points from low to high phosphate respectively, 0.05 M, 0.04 M, 0.03 M and 0 NaCl; drop diameter 8-11  $\mu\text{m}$ ;  $T = 25.0^\circ\text{C}$ . Top drops have a negative mobility, bottom drops a positive mobility.

A second interesting feature of the droplet electrokinetic behavior is shown in Figures 4 and 5 where the dependence on drop size is illustrated. The mobility is a monotonically increasing function of drop diameter in all cases and appears to increase linearly with diameter over the size range accessible. In all but one case (bottom phase drops in Figure 4) linear extrapolation of the data predicts a finite mobility as the diameter approaches zero. These results may imply solid-like behavior as the drop size diminishes and surface tension effects become dominant, since solid particles would not exhibit size-dependent mobilities under these conditions [12]. Alternately, the dependence of drop mobility on diameter may be nonlinear and the mobilities may approach zero below the sizes studied in this work. In the exceptional case a change in sign in mobility is predicted at about 4  $\mu\text{m}$  diameter. No actual reversal was observed, however, and the source of the effect is obscure at present.

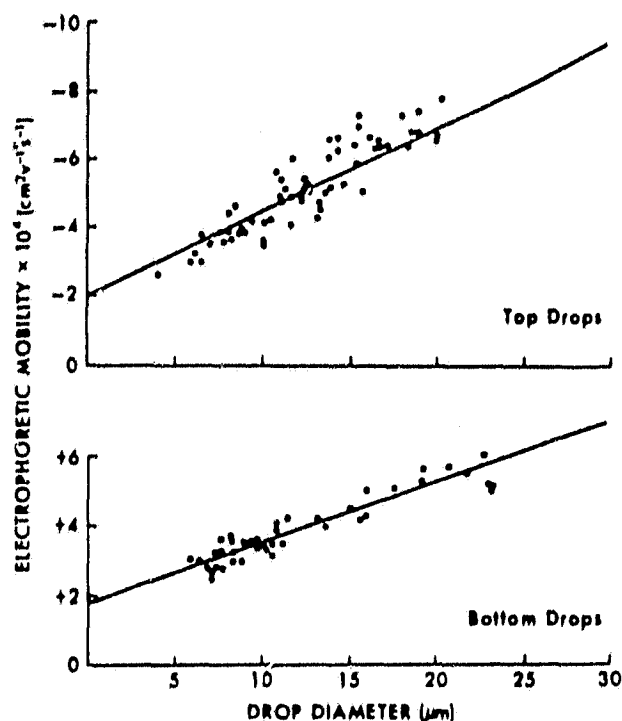


Fig. 4. Drop mobility vs diameter for the system containing 7.5% dextran T40, 4.5% PEG 6000 and 0.11 M Na phosphate, pH 7.5;  $T = 25.0^\circ\text{C}$ .

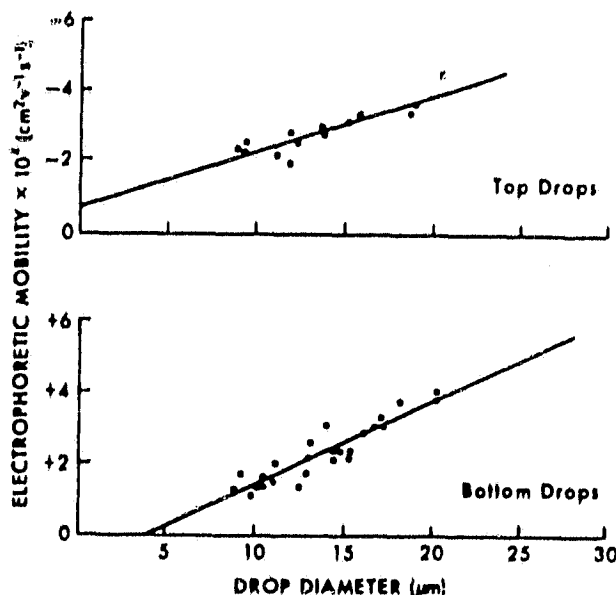


Fig. 5. Drop mobility vs diameter for the system containing 9% dextran T40, 6% PEG 6000 and 0.11 M Na phosphate, pH 7.5;  $T = 25.0^\circ\text{C}$ .

The studies illustrated, combined with the results of work in progress, make it clear that there is no simple relationship between the electrokinetic behavior and the distance of the system composition from the critical point. Probably two competing effects contribute to this behavior, mobility reduction due to viscosity increases for systems away from the critical point, and mobility increases associated with more extreme phosphate partition in systems with longer tie line lengths (Table 1). In spite of the unanticipated sign of the mobility, its magnitude appears to correlate with the value of the phosphate partition coefficient. In the systems examined to date, viscosity effects eventually take over as progressively more concentrated systems are used, however, and the mobilities are reduced (Figure 5).

The dependence of drop mobility on size is important with respect to the use of electric fields to produce phase localization in these systems. As an emulsified phase system begins to separate, droplet coagulation occurs. Since the settling velocity of isolated drops increases with the square of the radius [9], this leads to reasonably rapid separation. That the electrophoretic mobility of phase drops also increases - albeit more weakly - with increasing size suggests that qualitatively similar effects on phase separation might result from the application of an electric field. In fact, because of the magnitudes of the coefficients involved, with an applied field of  $4 \text{ v cm}^{-1}$  the electrophoretic velocity of, for instance, the bottom phase drops described in Figure 4 would be greater than the settling velocity for drops of up to 1.7 mm diameter.

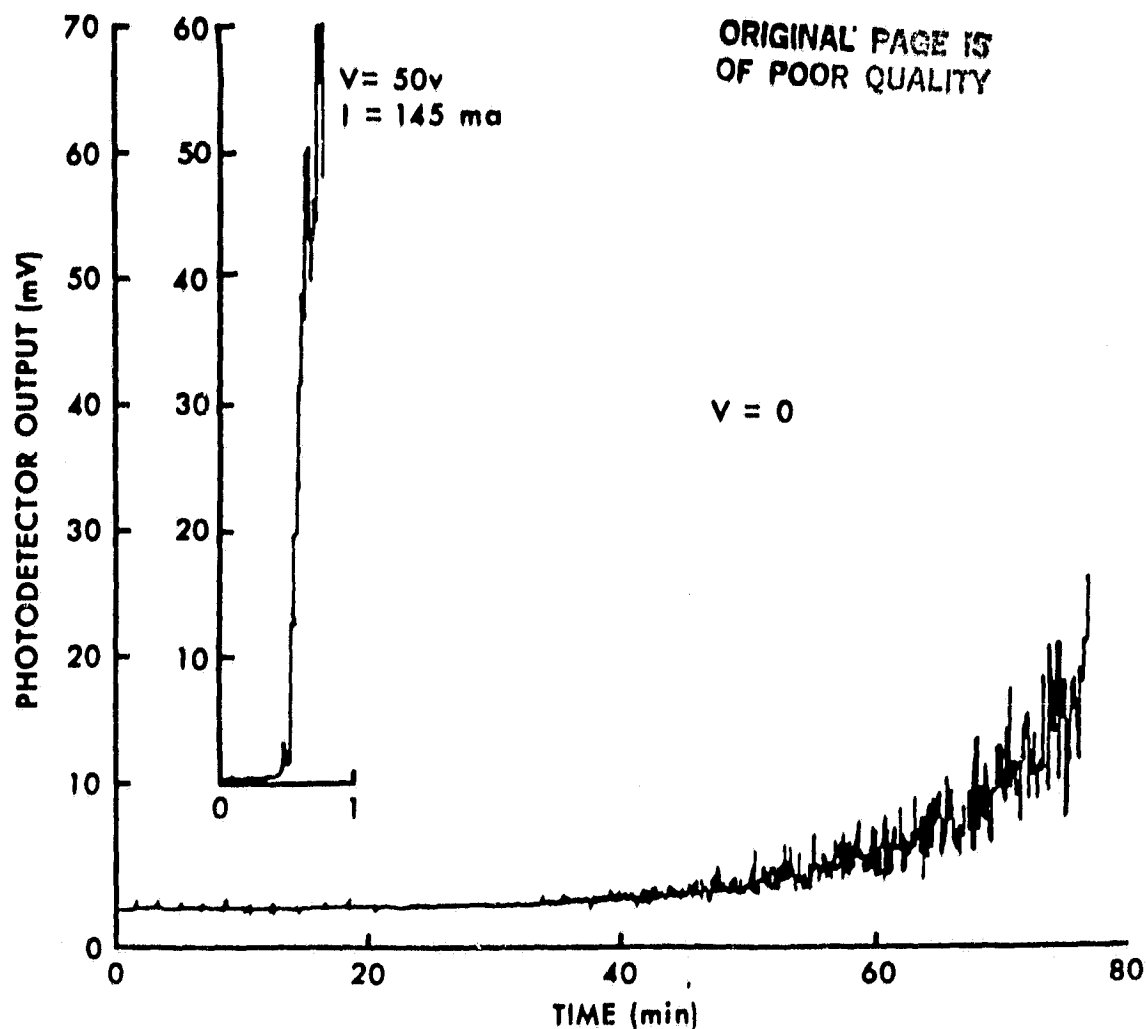


Fig. 6. Optical clearing of a 7.5% dextran T40, 4.5% PEG 6000, 0.11 M Na phosphate phase system in the absence (lower trace) and presence (inset) of an electric field of  $4.5\text{ v cm}^{-1}$ , top electrode positive. Note expanded time scale in inset.

Figure 6 illustrates the results of an experiment which demonstrates the feasibility of FDPS. The lower trace describes the optical clearing of a phase system containing 10% v/v bottom phase, 90% top, in the absence of an applied field. The inset shows the clearing observed when a field of about  $4.5\text{ v cm}^{-1}$  is applied, top electrode positive. The turbidity decreases between one and two orders of magnitude more rapidly under these conditions, suggesting that droplet electrophoresis has indeed resulted in a very rapid separation of the phases.

This interpretation is fully supported by measurements of the composition of solutions isolated from the top and bottom halves of the chamber following field application for various lengths of time. These measurements demonstrate that at a 1:1 top to bottom phase volume ratio the phases are effectively separated in less than one minute at field strengths  $4\text{ v cm}^{-1}$ . They also show that, with the optical path length and geometry

used, optical clearing occurs at a considerably later stage in the process than does the separation of most of the volume of each phase. Apparently the optical properties of the systems are dominated at later times by the presence of dispersions of very small drops of one phase in the other that do not represent a significant fraction of the total phase volume. Bulk phase separation occurs in our FDPS chamber with only an increase of a few percent in light transmission, therefore.

The above results demonstrate that dextran-PEG phase systems containing significant concentrations of phosphate buffer possess a variety of interesting equilibrium and electrokinetic properties. The physicochemical bases of the salt partition, and particularly the electrokinetic behavior of the phase systems, are not yet understood, although a theory describing phase drop electrophoresis is currently under development (see contribution of S. Levine in this collection). The electrokinetic properties are such that application of electric fields can enhance the rate of phase separation many fold. The prospects for applying FDPS to cell separation problems therefore appear to be good.

#### ACKNOWLEDGMENTS

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THE PARTITION OF SODIUM PHOSPHATE AND SODIUM CHLORIDE  
IN AQUEOUS DEXTRAN POLY(ETHYLENE GLYCOL) TWO PHASE SYSTEMS

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Running Title: Salt Effects on Two Phase Polymer Systems

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# ABSTRACT

It is known that the addition of phosphate buffer to two polymer aqueous phase systems has a strong effect on the partition behavior of cells and other particles in such mixtures. The addition of sodium phosphate to aqueous poly(ethylene glycol) dextran phase systems causes a concentration-dependent shift in the binodial on the phase diagram, progressively lowering the critical conditions for phase separation as the phosphate concentration is increased. Sodium chloride produces no significant shift in the critical point relative to the salt-free case. Accurate determinations of the phase diagram require measurements of the density of the phases; data are presented which allows this parameter to be calculated from polarimetric measurements of the dextran concentrations of both phases. Increasing polymer concentrations in the phase systems produce increasing preference of the phosphate for the dextran-rich bottom phase. Equilibrium dialysis experiments showed that poly(ethylene glycol) effectively rejected phosphate, and to a lesser extent chloride, but that dextran had little effect on the distribution of either salt. Increasing ionic strength via addition of 0.15 M NaCl to phase systems containing 0.01 M phosphate produces an increased concentration of phosphate ions in the bottom dextran-rich phase.

## INTRODUCTION

Polymer two phase aqueous systems have been used successfully for the separation of cells, cell organelles and macromolecules (1). Because the partitioning of such material has proved to be strongly dependent on the type and concentration of salt added to the system, the distribution of various salts themselves has been studied in systems containing dextran and PEG (2). It was found that polyvalent anions such as phosphate, sulfate and citrate partitioned preferentially into the dextran-rich bottom phase while halides partitioned nearly equally (2). As a result of the salt concentration difference thus set up, the addition of sulfate or phosphate to dextran/PEG phase systems induces an electrostatic potential difference between the two phases, the magnitude of which depends on the salt concentration (3,4). Furthermore, electrophoretic measurements of drops of one phase suspended in the other phase show that the electrophoretic mobility of the drops increases, for example, with increasing phosphate concentration (5). Since cell partitioning experiments in dextran/PEG phase systems often involve the use of phosphate to buffer the systems and to make them isotonic, we have carried out a more detailed examination of the effects caused by this salt.

## MATERIALS AND METHODS

The polymer stock solutions contained 25-30% w/w of either Dextran T40 ( $\bar{M}_w = 41,500$ ,  $\bar{M}_n = 26,800$ ; Pharmacia Fine Chemicals, Uppsala, Sweden; lot 7974), Dextran T500 ( $\bar{M}_w = 511,000$ ,  $\bar{M}_n = 191,600$ ; Pharmacia; lot 3447), PEG 6 ( $\bar{M}_w = 6,900$ ; Union Carbide, New York, NY; lot R-529-9104) or PEG 20 ( $\bar{M}_w = 20,000$ ; Union Carbide; lot 900 1S568182). The phosphate stock solutions were made by mixing aqueous solutions of 0.55 M sodium dihydrogen phosphate and 0.55 M disodium hydrogen phosphate of equal concentrations until the desired pH was reached.



The phase systems were made by mixing appropriate weights of polymer stock solutions with stock salt solutions and making up to the desired w/w concentration with twice distilled water in a graduated cylinder. The phase systems were allowed to separate at the desired temperature until neither phase showed visible turbidity. Following removal of all but 3 to 5 ml of top phase, the remaining top phase together with a few milliliters of the bottom phase was aspirated and discarded. Then the lower phase was drawn off into a syringe with a long needle from the bottom of the graduated cylinder, leaving behind the last 3 to 5 ml. The densities of the phases were obtained by pycnometry with an estimated error of  $\pm 0.2$  mg/ml.

The dextran concentrations in the phases and in the stock solutions were determined by polarimetry (Circle Polarimeter 0.05°, Carl Zeiss, Oberkochen, West Germany) with a precision of  $\pm 0.02\%$  w/v. The PEG concentrations in the stock solutions were calculated from the refractive indices of the solutions to a precision of  $\pm 0.1\%$  w/v. Since the refractive index increases linearly with the w/v concentrations of the polymers, as well as of the salt, the PEG concentrations in the phases were obtained by subtracting the respective increments for dextran and for the salt from the measured values. Dextran specific volume was determined pycnometrically. Tie line lengths were calculated from the top and bottom phase compositions according to:

$$TLL = [(PEG_t - PEG_b)^2 + (DEX_b - DEX_t)^2]^{1/2}$$
 where the subscripts refer to the PEG and dextran (DEX) concentrations in the top (t) and bottom (b) phases.

Phosphate concentrations were determined by the stannous chloride method (8) and were reproducible to within  $\pm 3\%$ . Because PEG precipitates and interferes with the assay, the organic material in the phases had to be removed by digestion in a mixture of concentrated nitric and sulfuric acids according to the procedure described by Taras (6). Occasionally the digestion did not completely oxidize the organic matter. In these cases additional concentrated nitric acid was added to the cooled reaction mixture and the

digestion was repeated. The chloride concentrations were determined with an Aminco-Cotlove automatic chloride titrator (American Instrument Co., Silver Springs, MD) and were reproducible to within  $\pm 1\%$ .

For the equilibrium dialysis experiments, dialysis tubing (Spectra/Por 6, molecular weight cutoff 2000, 64 mm diameter; Spectrum Medical Industries, Inc., Los Angeles, CA) was enclosed in a pouch of nylon netting to provide mechanical support. The tubing was filled with 1 to 2 ml of 40 to 50% w/w polymer stock solutions. The dialysis was carried out for 24 to 36 hours against 1 l of 0.11 M or 0.22 M sodium phosphate, pH 7.5, or 0.15 M NaCl, with the salt solutions changed once. Neither the addition of phosphate to the polymer stock solutions nor the increase of the dialysis period to 48 hours had significant effect on the results.

All measurements were carried out at room temperature in an air conditioned laboratory,  $22 \pm 1^\circ\text{C}$ .

## RESULTS

The optical methods used to determine the polymer concentrations provide w/v concentrations. To derive the % w/w values the densities of the phases have to be known. Figures 1 and 2 show that both the dextran concentration versus density of the bottom, dextran-rich phase and the difference in dextran concentration versus the difference in density between the two phases follow linear relationships. Similar linear relationships are found if the PEG concentration of the top phase is plotted versus the bottom phase density or the difference in PEG concentration is plotted versus the density difference between the phases, but not if the bottom phase dextran or the top phase PEG concentration is plotted versus the top phase density. In the top phase the density changes only slightly with changing polymer concentrations. For example, in systems

containing no salt the highest density of 1.026 g/ml was found in the system closest to the critical point. With increasing total polymer concentration the top phase density first decreases, reaching a minimum of 1.021 g/ml in 12.2% w/w Dextran T40 and 3.5% w/w PEG 6, then increases slightly to 1.022 g/ml at the highest polymer concentration investigated (14% w/w Dextran T40, 5% w/w PEG 6). The linear relationships given in Figs. 1 and 2 can be used to calculate the densities of all phases in the systems studied.

Figure 3 shows the binodials of the phase diagrams for Dextran T40/PEG 6 systems at different salt concentrations. The binodials for the systems with 0.15 M NaCl and without any salt added are so close to each other that only one line was drawn.

One parameter which can be used to describe the degree of phase separation is the length of the tie line which joins the two points on the binodial which describe the composition of the two phases. It is seen from Fig. 4 that as the polymer system approaches the critical point the tie line length is increasingly influenced by added salt.

Figure 5 shows that with increasing tie line length, phosphate partitions increasingly into the bottom, dextran-rich phase. The linear regression analyses for systems containing 0.11 M and for 0.22 M phosphate yield essentially parallel lines. That the lines are distinct indicates that the phosphate concentration, or ionic strength, influences the partitioning, the distribution becoming more one-sided the greater the amount of phosphate in the system. This is confirmed by the phosphate distribution in systems with 10 mM phosphate, pH 7.5, the values of which are all above those of systems with higher phosphate concentrations but having the same tie line length. However, if the ionic strength is increased with an indifferent salt, in this case 0.15 M NaCl in addition to 0.01 M phosphate, the top/bottom ratio drops slightly below that

for 0.22 M phosphate.

In some systems containing 0.15 M NaCl and 0.01 M sodium phosphate, pH 7.5 (5, 6, 6.5 or 7% w/w Dextran T500 and 4% PEG 6), the chloride concentrations in the top and bottom phase were determined. In all cases the top/bottom concentration ratios fluctuated around  $1.00 \pm 0.03$ .

To examine the contributions of the two polymers in determining phosphate partitioning the individual polymers were exhaustively dialyzed against various concentrations of phosphate at pH 7.5. The phosphate concentrations inside and outside the dialysis bag were then determined (Table I). For the calculation of the salt concentration ratio inside/outside, the inside concentration value was calculated based on the volume of water present, excluding that of the polymer. The volume occupied by the polymer was calculated using a specific volume for dextran, measured pycnometrically and in agreement with Edmond and Ogston (7), of 0.602 ml/g, and for poly(ethylene glycol), 0.837 ml/g (8). The resulting low concentration ratios for PEG 6 show the strong influence of poly(ethylene glycol) on the partitioning of phosphate. Dextran, however, yields an inside/outside ratio of close to one. Increasing PEG concentration produces more one-sided phosphate partitioning which increases in magnitude linearly with polymer concentration (Fig. 6). Within the accuracy of the measurements no clear dependence on PEG molecular weight or on total phosphate concentration was detected. Dialysis of the polymers against sodium chloride results in a lower salt concentration inside the dialysis bag than outside for both dextran and PEG 6. The inside/outside ratios for both polymers differ somewhat, with the PEG 6 value being lower than the dextran value.

## DISCUSSION

It is evident from the results presented above that the combinations of mono- and dibasic phosphate salts necessary to provide a physiological pH can have strong, concentration-dependent effects on the properties of dextran/PEG aqueous two phase systems. Increasing phosphate concentration moves the critical points towards lower dextran concentrations, whereas the PEG concentration at the critical point remains nearly unchanged (Fig. 3). The effect of this shift on the tie line length is dramatic near the critical point (Fig. 4), but becomes progressively less obvious in systems with longer tie line lengths, even though phosphate partitions strongly in favor of the dextran-rich phase under these conditions (Fig. 5). Apparently the polymer concentrations are sufficiently high that the PEG-dextran interactions dominate in determining the compositions of the phases (for a TLL of 25% w/w the dextran and PEG monomer concentrations are greater than 5 and 12 times those of the salt, respectively, on a molar basis). The addition of 0.15 M NaCl alone to the phase systems causes only minor changes, a slightly different phase composition yielding a much smaller elongation of the tie lines and a small shift in the critical point. NaCl therefore behaves as an indifferent salt in these systems. This is not because NaCl does not interact with either polymer in solution, however. The data in Table I shows that pure PEG and dextran solutions both effectively reject NaCl to a significant extent, PEG being the more effective of the two. In phase systems, in which the bottom phase dextran concentration is greater than the top phase PEG concentration, these effects apparently are balanced, the result being that no significant preferential partition of NaCl is observed.

Because of its recognized importance in affecting cell partition in these systems, the distribution of phosphate between the phases was of particular

interest. Phosphate partition out of the PEG-rich top phase was observed which increased with increasing tie line length phosphate concentration and ionic strength (Fig. 5). The equilibrium dialysis results imply the rejection of phosphate by PEG, with little contribution to the net partition from dextran. This idea is strongly supported by Fig. 6, in which the data from both the equilibrium dialysis and partition experiments are combined. It is seen that, if the difference in PEG concentration between the phases is used as the independent variable, the phosphate partition data from Fig. 5 lies within the scatter of the equilibrium dialysis results. That is, dextran apparently has no effect on the partition of phosphate, in spite of its high concentration in the systems. There is therefore no evidence to support the suggestion (2) that the ionic partitions observed in the phase systems result from the binding of ions to either polymer. Rather, support has been obtained from electrokinetic studies on cells to which dextran and PEG was adsorbed for anion exclusion by the polymers (9). The rejection of ions by PEG has also been observed in NaCl/PEG mixtures by Baldwin et al. (10), albeit at higher temperatures. These workers also observed an increase in the magnitude of the effect with increasing polymer concentration.

The mechanism responsible for the salt rejection by PEG is not known. It is evident from these and other (2) studies that it is the anion which is responsible for the effect, as varying the cationic species produces only small effects. It is possible that water bound to the ether oxygen may be effectively unavailable to participate in the hydration shell of the phosphate. Alternatively, and perhaps more likely, the hydrophobic ethylene group may induce water structures which are less readily able to hydrate phosphate than bulk solvent. It seems clear from the linearity of the polymer concentration dependence of phosphate partition over a wide range of PEG concentrations (Fig. 6), from the

lack of molecular weight dependence and from the activity of even very low molecular weight species in the work of Baldwin et al., that the incompatibility is a local effect involving the interaction of the anion with a relatively small oligomer. Other physicochemical techniques will have to be applied if the mechanism is to be better understood.

The dependence of phosphate partition on the concentration of phosphate and/or NaCl is also of interest (Fig. 5). While the effects are not large, it is clear from the error estimates of the data presented that phosphate partition out of the PEG-rich phase into the bottom phase increases with increasing ionic strength. That the dependence was not detected in the equilibrium dialysis experiments may be due to the relatively narrow range of high phosphate concentrations studied (Fig. 6). The effects of phosphate on TLL, which can be thought of as the reciprocal of the salt partitions observed, did not change much between 0.11 and 0.22 M phosphate (Fig. 4). The anion rejection from the top into the bottom phase can be thought of as a type of Donnan distribution, although the usual situation of ion binding to a macromolecule which is restricted to a particular phase or region of the solution (11) is reversed here. Nonetheless, the ion distribution which results will set up a Donnan potential. Increasing ionic strength suppresses this potential through a screening mechanism, thus allowing more one-sided salt partitions to occur (11). These are the qualitative effects seen at the highest TLL in Fig. 5. Why the 0.15 M NaCl increases the 0.01 M phosphate distribution to a disproportionately large degree is not clear, however.

#### ACKNOWLEDGEMENTS

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### FIGURE LEGENDS

Fig. 1: Dependence of the buoyant density of the bottom phase on the dextran concentration of the bottom phase at 22°C in aqueous phase systems of dextran T40 (closed symbols, solid lines or dextran T500; open symbols, dashed lines) with no salt added (o,●), with 0.15 M NaCl (▽,▼), with 0.11 M phosphate, pH 7.5 (□,■) and with 0.22 M phosphate, pH 7.5 (Δ).

Fig. 2: Dependence of the difference in buoyant density on the difference in dextran concentration between the two phases at 22°C. Symbols as in Fig. 1. The solid line represents the linear regression for all values of no salt and NaCl, the dotted line for all values of 0.11 M phosphate and the dotted dashed line for all values of 0.22 M phosphate.

Fig. 3: Phase diagram of aqueous phase systems of dextran T40 and PEG 6 with no salt added (o,●), with 0.15 M NaCl (▽,▼), with 0.11 M phosphate, pH 7.5 (□,■) and with 0.22 M phosphate, pH 7.5 (Δ,▲). Each open symbol represents the critical point which is the intersecting point between the binodial and the line through the midpoints of the tie lines of the same binodial. T = 22°C.

Fig. 4: Change of the TLL with total concentration of sodium phosphate, pH 7.5 in: 7.5% w/w dextran T40, 4.5% w/w PEG 6 (●); 10.7% dextran T40, 4.3% PEG 6 (▲); 14% dextran T40, 5% PEG 6 (■); and the concentration of sodium chloride in 7.5% dextran T40, 4.5% PEG 6 (o).

Fig. 5: Phosphate concentration ratio, top/bottom, at different total phosphate concentrations as a function of the length of the tie line (% w/w) in dextran T40/PEG 6 two phase systems with 10 mM phosphate (o), 0.01 M phosphate and 0.15 M NaCl (●), 0.11 M phosphate (■), and 0.22 M phosphate (▲) total concentrations at 22°C. All systems had a pH of 7.5.

Fig. 6: Phosphate concentration ratio,  $\text{phosphate}_t/\text{phosphate}_b$ , after dialysis of PEG 6 (o,●) or PEG 20 (Δ,▲) at 22°C against 0.11 M (open symbols) or 0.22 M sodium phosphate pH 7.5 (closed symbols). The line represents the linear regression analysis for all circles and triangles. In addition the partition coefficients of phosphate,  $\text{phosphate}_{\text{top}}/\text{phosphate}_{\text{bottom}}$ , of the systems in Fig. 5 with 0.11 M (□) and 0.22 M phosphate (■) are plotted versus the difference in the % w/w PEG-concentration,  $\Delta\text{PEG}$ , between top and bottom phase.

**Table I:** Equilibrium concentration of sodium phosphate, pH 7.5 ( $\text{PO}_4$ ) or sodium chloride ( $\text{NaCl}$ ) inside and outside dialysis sacs.

System	% w/w Polymer	Measured Salt Concentrations (mM)		<u>Inside</u> <u>Outside</u>
		Inside*	Outside*	
I. 0.11 M PO <sub>4</sub>				
Dextran T500	10.65	101.6±1.2	106.9±2.0	1.019
Dextran T500	12.02	104.2±1.2	111.7±1.1	1.011
H <sub>2</sub> O	-	109.1±0.4	107.4±1.0	1.016
II. 0.22 M PO <sub>4</sub>				
Dextran T500	11.34	199.4±0.3	215.1±0.8	0.995
III. 0.15 M NaCl				
PEG 6	6.69	129.2±0.1	145.5±0.1	0.952
PEG 6	6.25	133.6±0.2	149.1±0.1	0.946
PEG 6	12.02	116.6±0.1	149.1±0.1	0.872
PEG 20	3.50	145.6±1.1	149.0±0.5	1.007
PEG 20	3.92	142.5±1.0	149.0±0.5	0.989
PEG 20	4.70	139.8±0.7	149.0±0.5	0.977
Dextran T500	13.60	134.9±0.3	151.3±1.0	0.976

\*  $\pm$  Standard error of the mean.

Fig. 1

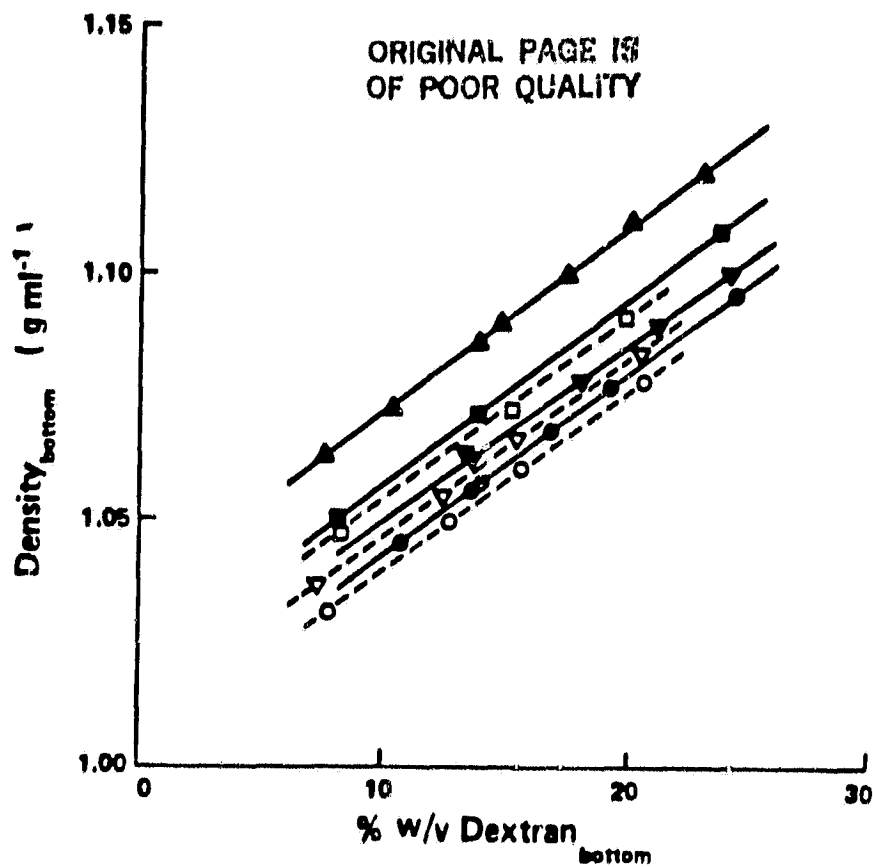


Fig. 2

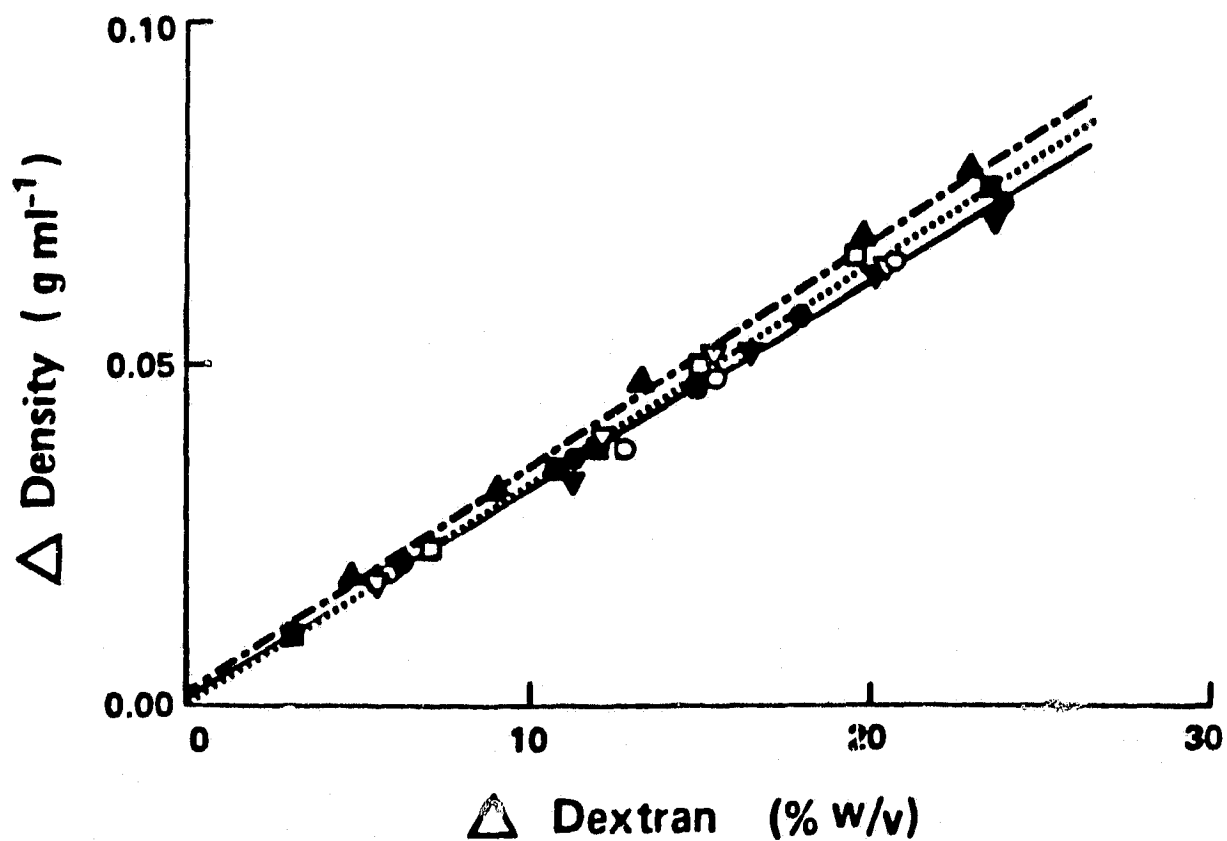


Fig. 3

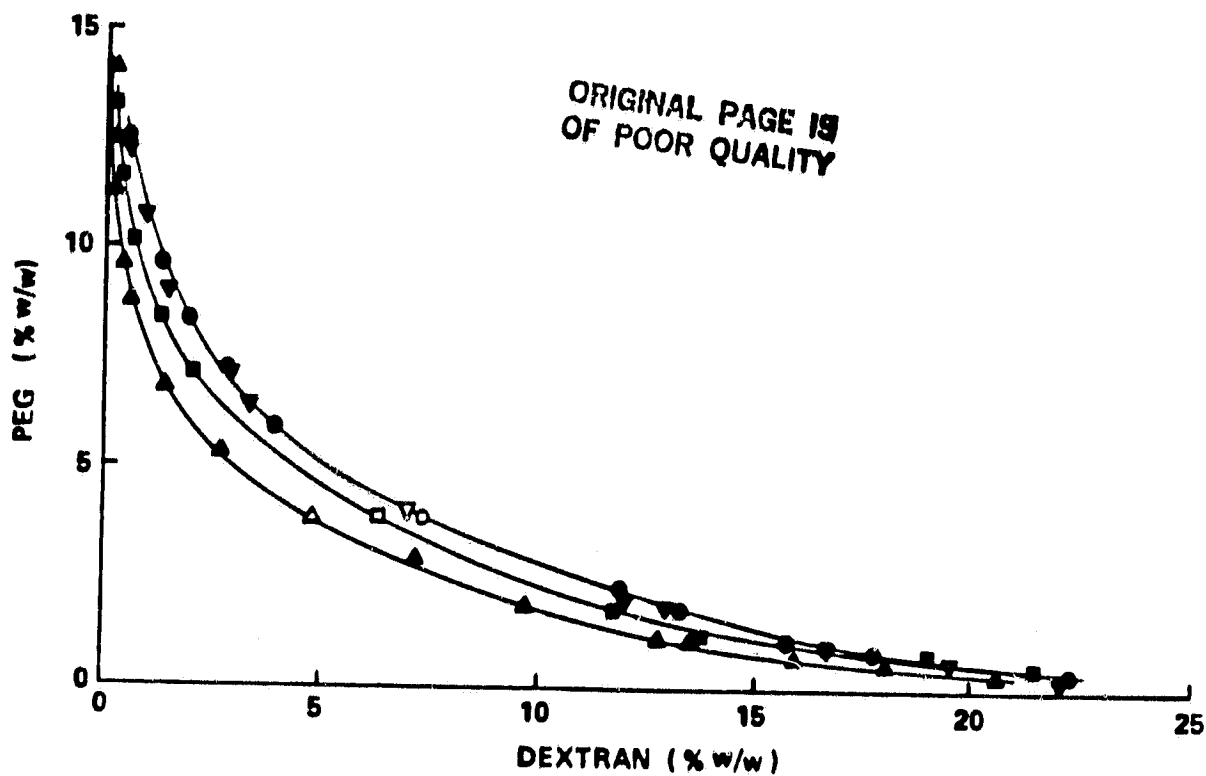


Fig. 4

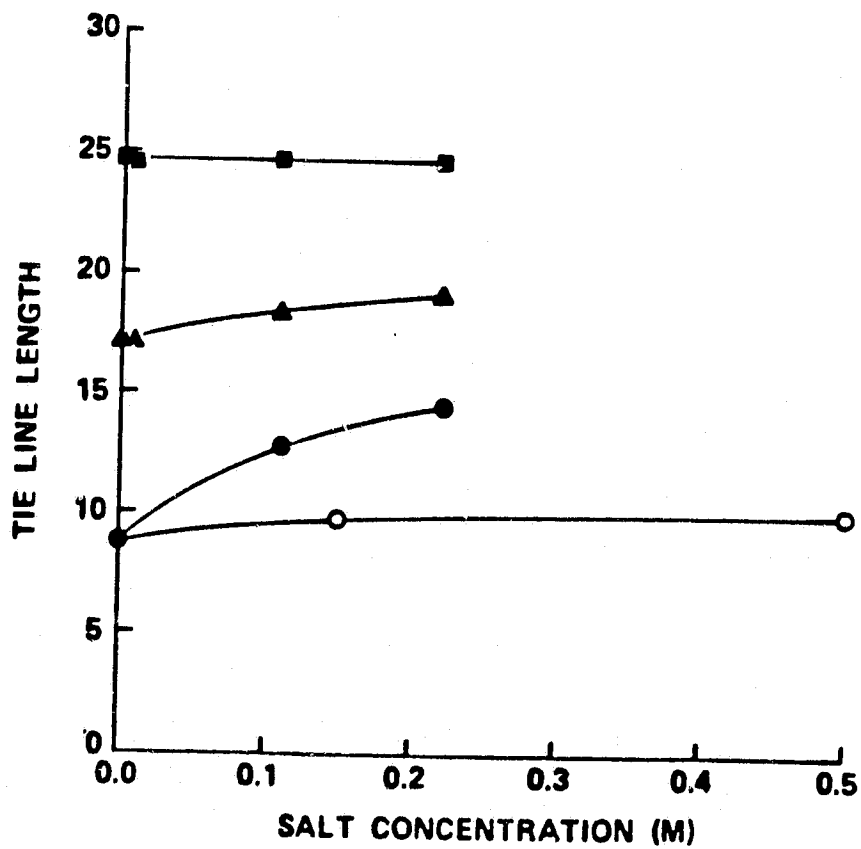


Fig. 5

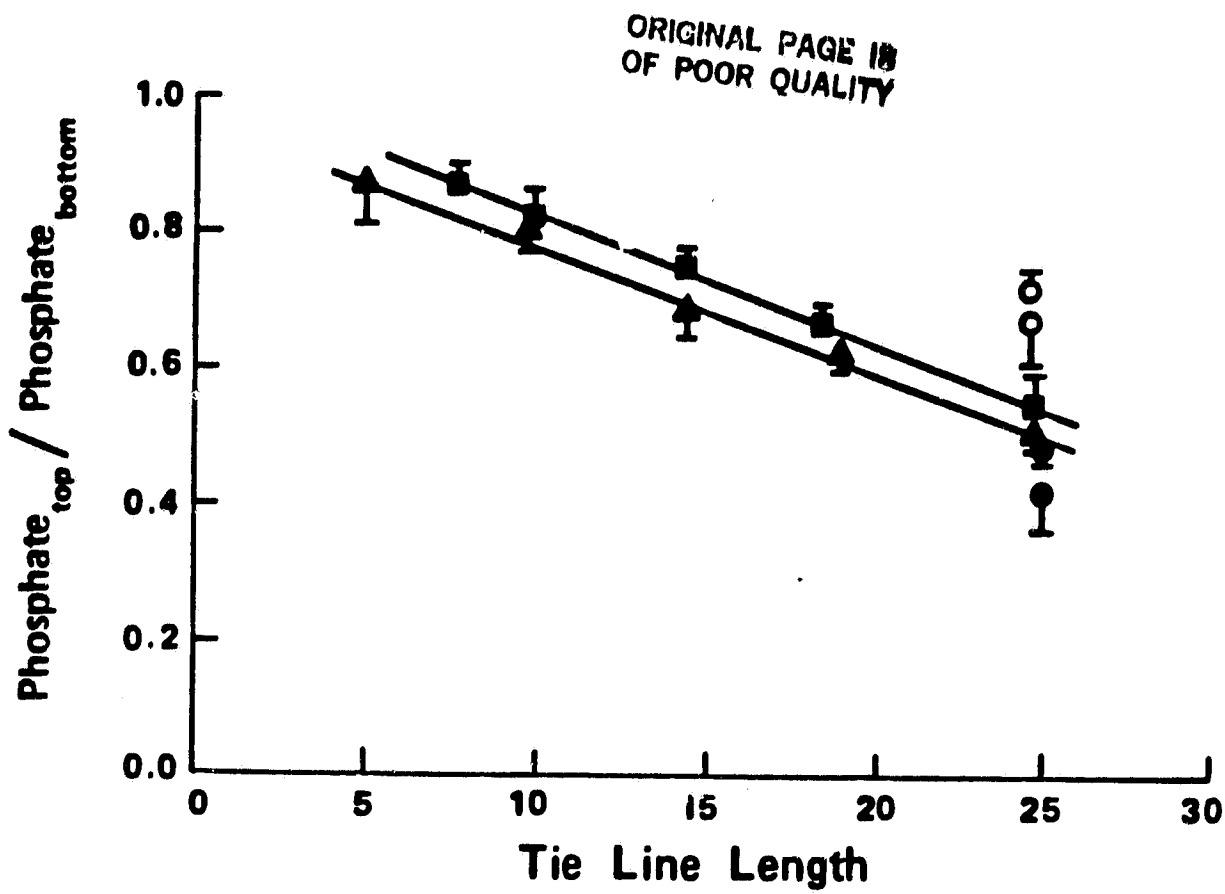
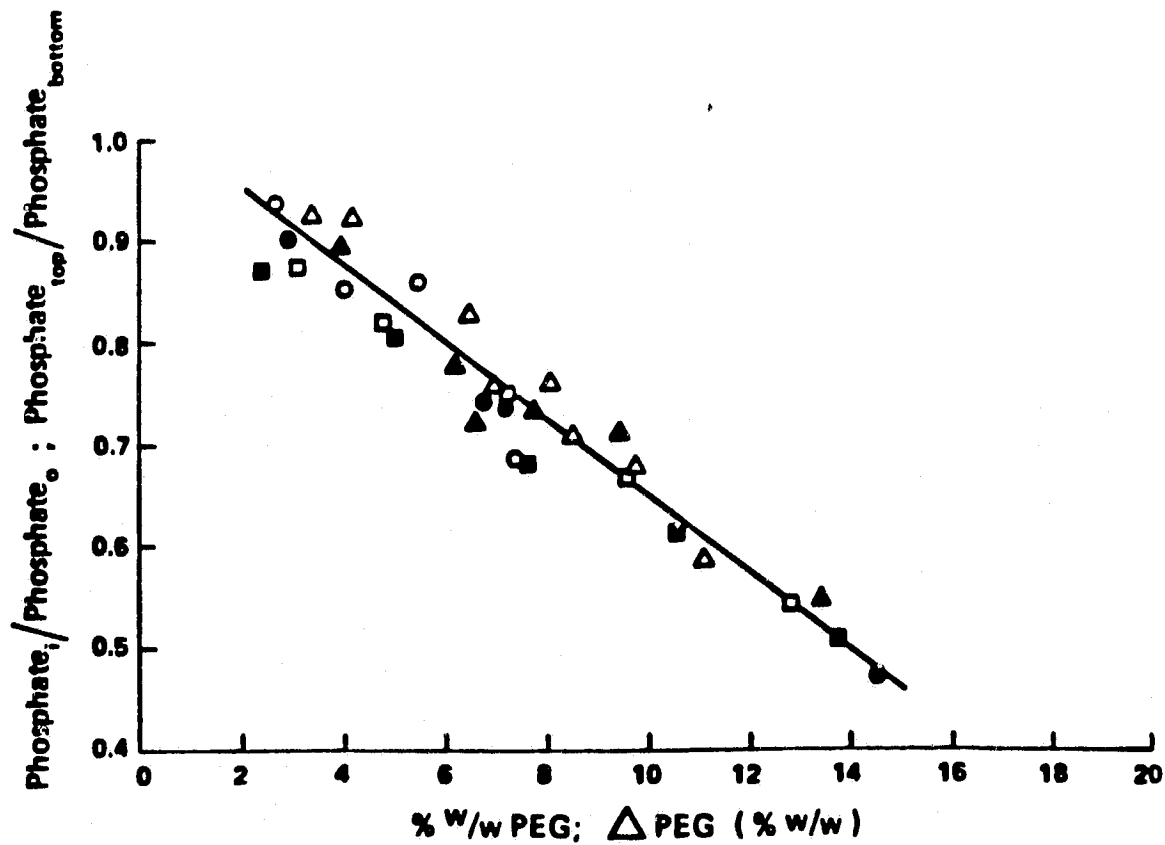


Fig. 6



THE EFFECTS OF SALTS ON THE INTERFACIAL TENSION OF  
AQUEOUS DEXTRAN POLY(ETHYLENE GLYCOL) PHASE SYSTEMS

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ABSTRACT

The use of the rotating drop technique to measure the interfacial tension,  $\gamma$ , of aqueous poly(ethylene glycol) (PEG)/dextran phase systems was studied and compared to the pendant drop method. The dependence of  $\gamma$  on the concentrations of the polymers, sodium phosphate and sodium chloride was investigated. The difference in polymer concentrations between the phases plotted against  $\gamma$  on a log-log scale yields straight parallel lines for the different salt concentrations. The tension tie-line length relationship therefore obeys a power law. The influence of temperature on  $\gamma$  was unusual in that for equal tie-line lengths the tension is lower at 6°C than at 22°C in systems containing 0.11 M phosphate.



## INTRODUCTION

Phase separated two polymer aqueous systems, when suitably buffered, act as effective partition media for biological colloids (1). The interfacial tension between the phases,  $\gamma$ , is an important determinant influencing the partitioning behavior of particles or cells because such materials usually distribute themselves between the interface and one of the two bulk phases, leaving the other phase empty of particles (1). The degree to which interfacial adsorption will occur increases with increasing  $\gamma$  because of the greater decrease in free energy associated with loss of free liquid/liquid interfacial area between the native phases. Ryden and Albertsson (2) and recently Schürch, Gerson and McIver (3) reported a strong dependence of the interfacial tension between the two phases on polymer concentration, with values as low as 0.5  $\mu\text{N/m}$ . As shown in the preceding paper, dextran/poly(ethylene glycol) two phase systems are strongly affected by the presence of phosphate (4,5). The purpose of the present work, therefore, was to investigate the dependence of interfacial tension on both phosphate and polymer concentrations and also on temperature. The results obtained for systems containing either sodium chloride or no added salt are compared with those for phosphate systems.

## MATERIALS AND METHODS

The preparation of the dextran/poly(ethylene glycol) systems is described in the preceding paper.

The rotating drop method was used to determine the interfacial tension between the phases, as first described by Vonnegut (6) and analyzed in detail by Princen et al. (7). In this technique a small drop of the less dense phase is injected into a glass tube filled with the more dense phase. Rotation of the tube about its axis of symmetry produces centrifugal forces which cause

migration of the drop to the center line of the tube. At increasing rotational velocities the drop becomes more and more elongated. If the rotational speed, the difference in density between the drop and the surrounding medium, the drop volume, and the length of the drop are known the interfacial tension can be calculated (7) provided that gyrostatic equilibrium is attained (8).

An interfacial tension apparatus was constructed according to the descriptions of Vonnegut (6) and Princen et al. (7). Rotation of the sample tube, having an inner diameter of 7 mm and a length of 100 mm, is accomplished by means of a variable speed DC motor (Type DPM-4330E, Bodine Electric Co., Chicago, IL) linked to the tube assembly mounted on the bed of a miniature precision lathe (Model 334 B400, Jensen Tools & Alloys, Tempe, AZ). Figure 1 shows a close-up of the rotating glass tube. In order to provide accurate centering of the tube it is held at both ends by spherically shaped end pieces, the diameters of which are slightly larger than the inner diameter of the tube. Fluid is maintained in the tube by two stainless steel plugs, each held in place by a pair of O-rings. One of the plugs is drilled to produce a 1.3 mm diameter hole through which the drop of the lighter phase is injected via a microsyringe (Hamilton, Reno, NV). In the present experiments the injected volume was varied between 1 and 50  $\mu$ l depending on the anticipated interfacial tension. Immediately after injection the tube is mounted and allowed to rotate slowly (100-150 rpm) until the drop reaches the center of the tube. It is often possible to merge drops at high speed of rotation and then gradually slow down until the desired condition is reached. The length of the drop is measured by means of a traveling microscope at a magnification of 100X. The apparent drop diameter at any point can be measured with a filar micrometer (American Optical Co., Buffalo, NY)

built into the 10X eyepiece of the traveling microscope. The true diameter, corrected for the cylindrical lens effect, is calculated using the calibration factor determined by measuring the diameters of rods of known size centered inside the glass tube containing a bottom phase. The rate of rotation is measured using a digital frequency counter (Type 5381A, Hewlett-Packard, Palo Alto, CA) which can be read to an accuracy of 0.1 rpm. The rotation rate can be varied up to 2500 rpm; all measurements were performed between 300 and 2500 rpm. The construction of the stainless steel plugs closing the tube allows the replacement of the drop without the need to exchange the bottom phase. After an experiment the tube is placed into a vertical position with the perforated plug at the top until all the top phase has reached the top. The top phase is then removed through the plug hole by pushing one of the plugs slightly. Room temperature measurements were made in an air conditioned laboratory where the temperature was  $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . Measurements at  $6^{\circ}\text{C}$  were made in a cold room at  $6^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . Because of the difficulty in injecting a microliter drop of known volume, the drop volume was determined from measurements of drop length and diameter, applying the tables of Princen et al. (7). The accuracy with which the length ( $\pm 20 \mu\text{m}$ ) and diameter ( $\pm 5 \mu\text{m}$ ) could be estimated placed a practical lower limit of about  $0.5 \mu\text{l}$  on the volumes used.

For selected systems, interfacial tensions were also measured by the pendant drop method (9,10). Drops of the more dense phase were extruded into the less dense phase via a micrometer-controlled syringe (Hamilton) to which was coupled a glass micropipette, drawn on a conventional pipette puller then broken and heat polished to a tip inside diameter in the range 15-35  $\mu\text{m}$ . Drops were hung in a chamber constructed from a microscope slide, spacers and glass cover slip, the whole assembly being mounted in a

thermostatted water bath into which a horizontal microscope barrel equipped with a 40X water immersion objective protruded. A video camera with a 100 mm lens, 19 inch monitor and a video tape recorder were used to view and record the drops. Dimensions of drops stable for at least 1 min were either measured directly off the screen or obtained using video calipers (Model 305, Vista Electronics, La Mesa, CA), calibration being derived from a stage micrometer imaged in the water bath.

## RESULTS

The results of a series of interfacial tension measurements on the same phase system are shown in Fig. 2, where the apparent tension, calculated utilizing the measured drop length and diameter at the mid-line of the drop, is plotted as a function of rotation speed for drops of various sizes. The effect illustrated is due to the progressively more extreme deviation of drop geometry from a cylindrical towards a dumbbell form as drop volume is increased, an effect which is obviated by increasing the rotation speed. It is also seen that if an effective drop radius, defined by:

$$Y_{\text{eff}}^2 = 1/3(Y_{\text{max}}^2 + Y_{\text{max}} Y_c + Y_c^2)$$

where  $Y_{\text{max}}$  = maximum value of drop radius,  $Y_c$  = radius at center line, is used as the basis of the  $\gamma$  calculation, the results for large drops are improved somewhat. The minimum in the apparent value for  $\gamma$  for larger drops occurs when the ratio of the drop length to the central diameter is about 6. For smaller drops the value of  $\gamma$  is essentially independent of the speed of rotation and  $\gamma$  measured by this spinning drop technique is in reasonable agreement with values obtained by the pendent drop method.

In Fig. 3  $\log \gamma$ , measured at room temperature, is plotted against the log of the tie line length (TLL; length of the line connecting the top and bottom phase system compositions on the phase diagram) for dextran/PEG

systems containing various concentrations of salt. Figure 4 shows similar plots are obtained if the  $\log \gamma$  data are plotted against the log of the difference in dextran concentration between the phases. Similar data, obtained at 6°C, are presented in Table I for systems containing 0.11 M phosphate. The linearity of all the log-log plots is analyzed in Table II where the linear regression coefficients are all seen to exceed 0.99, indicating that  $\gamma$  depends upon the polymer distributions into the system according to a power law, the coefficients of which are modified by temperature and by the nature and concentration of added salts. Plots of  $\log \gamma$  versus the log of the difference in PEG concentrations between the phases also show linear relationships for the salt concentrations. Since the systems containing 0.15 M NaCl or no salt added are not buffered, the possible influence of a pH different from 7.5, both on the polymer composition in the phases and on the interfacial tension, had to be considered. Therefore, some systems containing 0.01 M phosphate, pH 7.5, were investigated; the results are listed in Table III. Because in practical partition work phase systems are generally made up at constant total polymer concentration, and the salt varied, the specific characteristics of a few such systems are listed in Table IV. In addition, two systems employing a higher molecular weight dextran fraction are included.

#### DISCUSSION

As has been discussed in detail by Manning and Scriven (8), the spinning drop method can exhibit considerable complexities when very low values of  $\gamma$  are being estimated. Secondary flows are present in these systems which are apparently sufficient to deform the larger drops out of the expected cylindrical shape into a symmetrical form with a minimum vertical radius at the center line and two maximum radii near each end. Increasing the rotation

speed can overcome this tendency, but for the larger drops the time necessary to come to an equilibrium shape can be prohibitively long. Hence, the smaller drop sizes give more reliable results and are easier to work with. In these cases the volumes have to be determined from the linear dimensions, however, necessitating the use of an instrument capable of length measurements along both principal axes. In systems near the critical point, measurements on small drops should be taken as rapidly as possible after loading the drop since the tension and phase composition are relatively sensitive to temperature fluctuations, particularly any heating originating from the bearings or motor. Heating from the microscope lamp can be avoided by interposing a heat filter (water cuvette containing cupric sulfate) between it and the rotating tube. With these provisos in mind, however, it is evident from the results in Fig. 2 that reliable values can be obtained that agree with those made by the pendent drop technique which are not subject to these particular difficulties. The latter technique suffers from other limitations, however (9), and it is our opinion that for measurements on two phase polymer systems the spinning drop method is easier to use and more reliable.

The strong influence of phosphate on the phase composition of the PEG/dextran system was discussed in the previous paper (4). Figures 3 and 4 show that the interfacial tension, too, is strongly affected by this salt. The log-log plots of tension vs tie line length show that phosphate increases the tension in a concentration-dependent manner. Moreover, this effect is not just due to the increase of TLL associated with phosphate incorporation, since the data for different phosphate concentrations lie on different lines. Phosphate therefore enhances the interfacial tension through an independent mechanism, presumably associated with the phosphate concentration gradient which exists

across the phase boundary.

Although NaCl also causes (small) increases in tie line length and therefore increases the interfacial tension (Table III), this salt has no independent effect on the tension, the dependence of  $\gamma$  on TLL in 0.15 M NaCl being indistinguishable from that obtained in the absence of salt.

The strictly linear relationship between  $\log \gamma$  and  $\log \text{TLL}$  seems to differ from the results of earlier work (2,3) in which  $\log \gamma$  was reported to depend linearly on TLL, implying an exponential relationship between the two. Clearly such dependence could not hold for  $\text{TLL} \rightarrow 0$ , however. In fact, if the results of Ryden and Albertsson (2) and Schürch et al. (3) are plotted as in Fig. 4, the fit is as good as or better than the published lines. Hence, it is concluded that  $\gamma$  follows a power law dependence on TLL with the coefficients given in Table II.

It is of interest that the power law dependence found here bears a considerable resemblance to the relationship found by Macleod (11) between the surface tension of a pure liquid near the critical temperature and the difference in density across the phase boundary,  $\Delta\rho$ . In this case it was found that  $\gamma$  varied as  $\Delta\rho^4$ . Since the tie line length is a measure of the composition difference across the interface, there is some analogy between the  $\Delta\rho$  and TLL. Hence, the similarity in the functional form of the dependence, and the magnitudes of the powers involved (3.5-4.2 vs 4) may be more than coincidental.

A comparison of Figs. 3 and 4 shows that substitution of the tie line length by the difference in dextran concentration between the phases yields very similar results for both the regression coefficients and the shift of the lines caused by increasing phosphate concentrations. This is explained by the very good proportionality found between the TLL and the difference in dextran concentration between the phases,  $\Delta\text{DEX}$ . The influence of 0.15 M NaCl and of 0.22 M phosphate is small, since the factor A in the relationship

$\Delta\text{DEX} = A \times \text{TLL}$  is 0.88 for the salt-free and NaCl systems, and 0.85 for 0.22 M phosphate. Since geometry demands that  $\text{TLL}^2 = \Delta\text{DEX}^2 + \Delta\text{PEG}^2$ , the observed proportionality between TLL and  $\Delta\text{DEX}$  implies that TLL is proportional to  $\Delta\text{PEG}$  as well. This relationship was less accurately verified experimentally, however, probably because of errors involved in the indirect determinations of PEG.

Although not studied exhaustively, the effects of changing temperature and dextran molecular weight were also noted during this study (Table I and IV). If systems of the same total composition are compared, the interfacial tensions increase with decreasing temperature, as is generally found for most liquid interfaces (12). The more relevant comparison, however, is between systems whose phase compositions differ to the same degree, i.e., those with the same TLL. It is clear from Table II that systems of equal TLL have a lower tension at the lower temperature. It is possible that this effect is due to the temperature dependence of the phosphate partition, which we have not measured, but this seems unlikely since the  $\log \gamma$ - $\log \text{TLL}$  line for the 0.11 M phosphate system at 6°C lies below the equivalent line for the system containing no salt at 22°C near the critical point.

Polymer molecular weight also had an independent effect on  $\gamma$ . It was found that increasing the dextran molecular weight from approximately  $4 \times 10^4$  to  $5 \times 10^5$  daltons increased the interfacial tension by about 50% at  $\text{TLL} = 11\%$  in 0.11 M phosphate and a similar effect was observed in 0.15 M NaCl. Both the above observations are relevant to the choice of systems for the separation of particle populations in these phase systems.

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## FIGURE LEGENDS

Fig. 1: Rotating drop of top phase suspended in bottom phase in glass tube of interfacial tension apparatus.

Fig. 2: The apparent interfacial tension of rotating drops with the following volumes: 1  $\mu\text{l}$  ( $\Delta$ ), 5  $\mu\text{l}$  ( $\bullet$ ), 15  $\mu\text{l}$  ( $\blacksquare, \square$ ), and 25  $\mu\text{l}$  ( $\nabla, \nabla$ ), as a function of rotation speed, for a phase system composed of 5% dextran T500, 4% PEG 6, 0.11 M phosphate, pH 7.5,  $T = 22^\circ\text{C}$ . Open symbols indicate values of  $\gamma$  calculated from the effective drop radius defined in the text. In the case of the drops with 1  $\mu\text{l}$  and 5  $\mu\text{l}$  volumes the calculation of  $\gamma$  was based on the length and on the average volume of each drop; the average volume was determined from the length and central diameter at different rates of rotation. The dotted horizontal line indicates the value obtained via the pendant drop method, the error bars indicating  $\pm 1$  S.D.

Fig. 3: Dependence of the interfacial tension on the tie line length at  $22^\circ\text{C}$  in systems containing dextran T40 and PEG 6 with no salt added ( $\bullet$ ), with 0.15 M NaCl ( $\nabla$ ), with 0.11 M phosphate, pH 7.5 ( $\blacksquare$ ) and with 0.22 M phosphate, pH 7.5 ( $\Delta$ ). The coefficient of variation ranges between 0.7 and 0.5%, with the large errors arising from the lower values of interfacial tension.

Fig. 4: Dependence of the interfacial tension on the difference in dextran concentration between the top and bottom phase at  $22^\circ\text{C}$ . The symbols are the same as in Fig. 3.

Table I: Properties of dextran T40/PEG 6-systems containing 0.11 M sodium phosphate pH 7.5 at 6°C. The polymer concentrations are listed in % w/w.

Total		Top phase		Bottom phase		TLL	$\gamma$ ( $\mu\text{N/m}$ )
DEX	PEG 6	DEX	PEG 6	DEX	PEG 6		
6.0	4.0	3.66	5.18	9.35	2.46	6.31	0.404
7.0	4.0	2.32	6.28	11.89	1.62	10.64	3.59
7.5	4.5	1.38	7.54	14.02	1.17	14.15	15.2
10.0	5.0	0.59	9.88	18.36	0.69	20.01	54.5
14.0	5.0	0.27	12.39	22.55	0.32	25.34	118

**Table II:** Coefficients for the linear regression equations used for fitting the lines for the data presented in Figs. 4 and 5 and Table I.

System \ Correlation	$\log \gamma = A + B \log \text{TLL}$			$\log \gamma = A + B \log (\Delta \text{DEX})$		
	A	B	$r^2$	A	B	$r^2$
no salt, 22°C	-3.13	3.64	0.996	-3.05	3.73	0.999
0.15 M NaCl, 22°C	-3.21	3.71	0.999	-3.09	3.78	0.999
0.11 M $\text{PO}_4$ , 22°C	-3.14	3.77	0.999	-3.01	3.86	0.999
0.22 M $\text{PO}_4$ , 22°C	-2.62	3.52	0.998	-2.49	3.64	0.998
0.11 M $\text{PO}_4$ , 6°C	-3.68	4.14	0.996	-3.56	4.22	0.997

Table III: Influence of 0.01 M sodium phosphate pH 7.5 and 0.15 NaCl in systems containing dextran T40 (DEX) and PEG 6 on the interfacial tension ( $\gamma$ ) and the tie line length (TLL) at 22°C. The polymer concentrations are listed in % w/w.

DEX	PEG 6	PO <sub>4</sub>	NaCl	$\gamma$ ( $\mu$ N/m)	TLL
9	4	-	-	4.94	11.53
9	4	+	-	4.70	11.27
9	4	-	+	4.84	11.45
9	4	+	+	7.51	12.45
14	5	-	-	84.2	24.73
14	5	+	-	84.5	24.62
14	5	-	+	82.7	24.67
14	5	+	+	95.0	24.96

**Table IV:** Properties of 7.5% dextran T40/4.5% PEG 6 (a) and of 5% dextran T500/4% PEG 6 (b) containing different concentrations of NaCl and of sodium phosphate pH 7.5 at 22°C. The polymer concentrations and tie line lengths are listed in % w/w. ND: not determined.

System	Salt	Top phase			Bottom phase			$\gamma$ ( $\mu\text{N/m}$ )	Critical point	
		DEX	PEG 6	DEX	DEX	PEG 6	TLL		DEX	PEG 6
a	-	3.96	5.95	11.93	2.31	8.76	2.11	7.3	3.9	
a	0.15 M NaCl	3.35	6.50	12.03	2.06	9.75	2.87	7.0	4.0	
a	0.50 M NaCl	3.09	6.82	11.85	2.03	9.98	3.27	ND	ND	
a	0.11 M $\text{PO}_4$	1.69	7.75	12.87	1.67	12.74	10.8	5.4	3.9	
a	0.22 M $\text{PO}_4$	0.62	8.79	12.80	1.17	14.37	31.1	4.8	3.8	
b	0.15 M NaCl	0.70	6.13	10.45	1.62	10.74	6.45	ND	ND	
b	0.11 M $\text{PO}_4$	0.38	6.42	10.29	1.48	11.07	9.19	ND	ND	

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Fig. 1

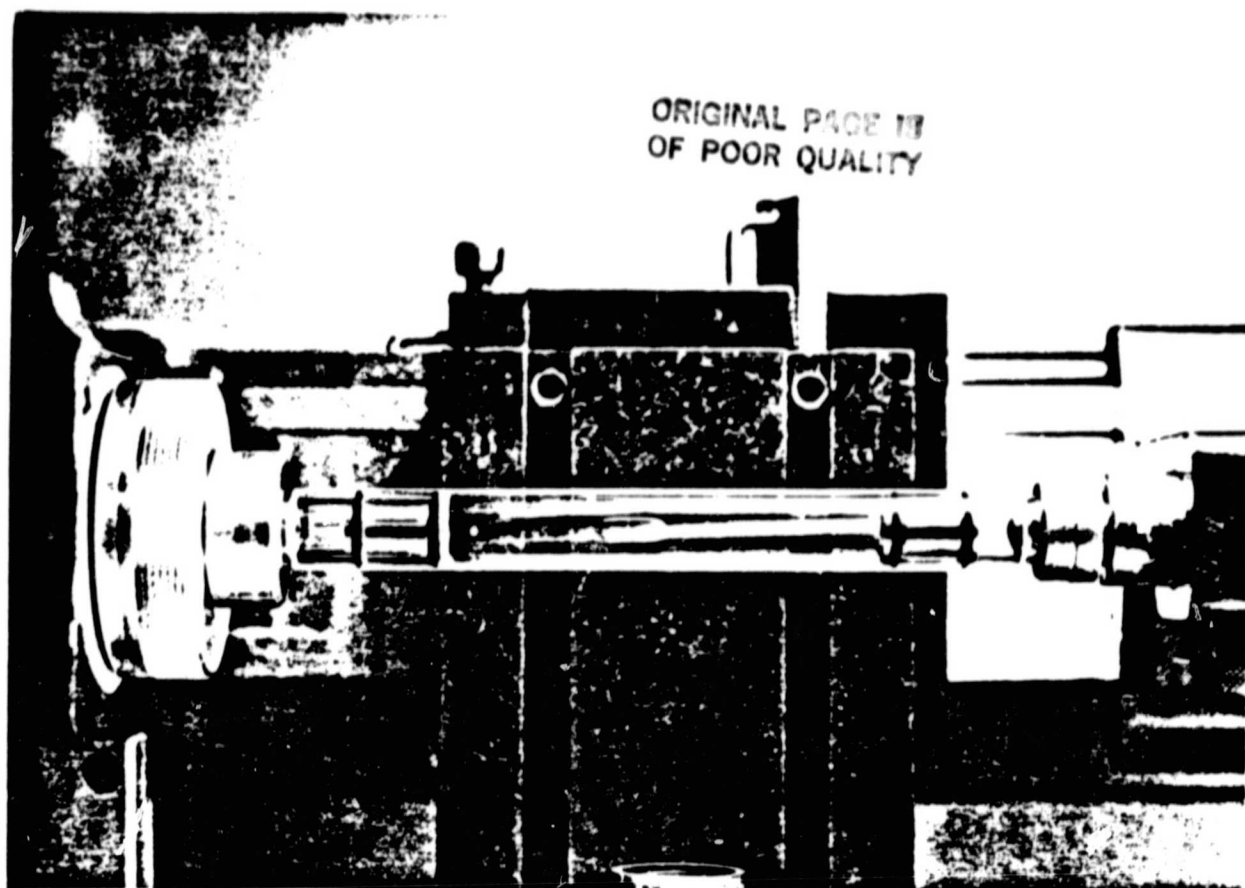
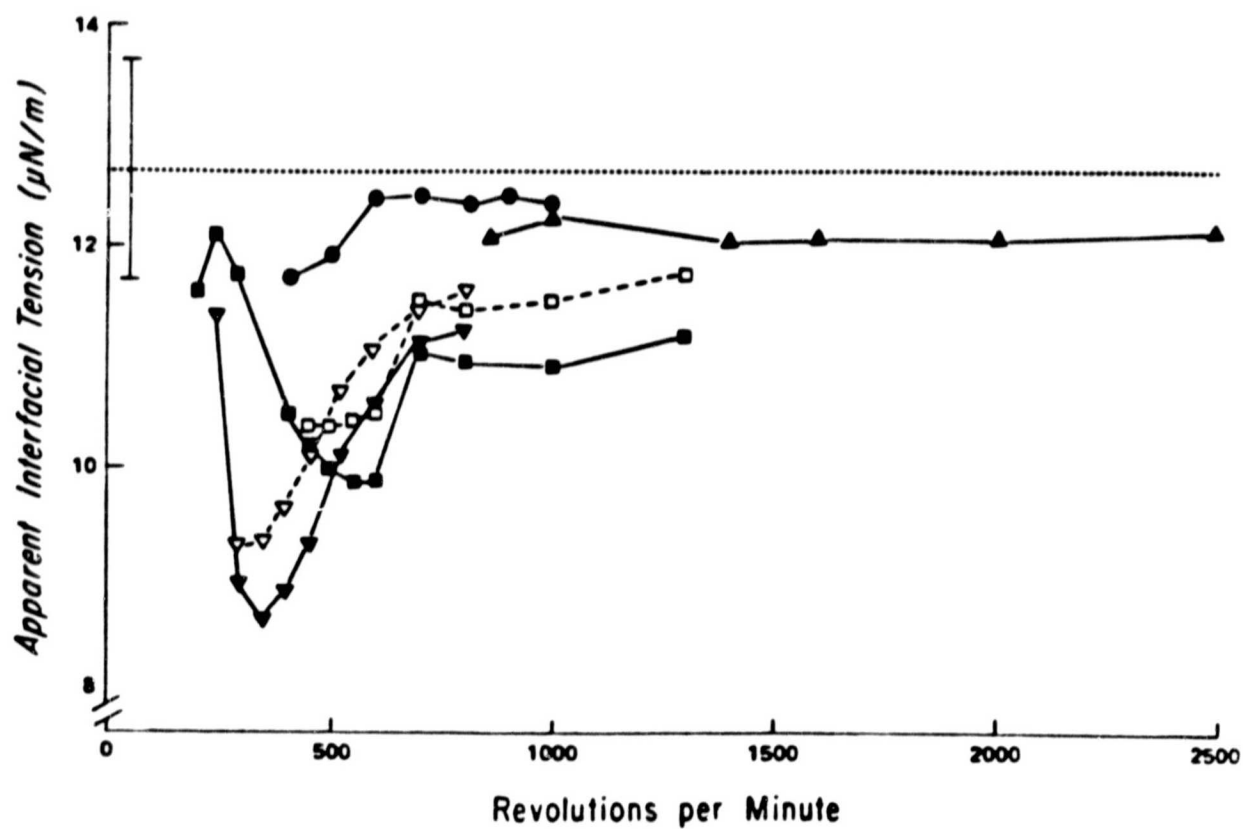


Fig. 2





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Fig. 3

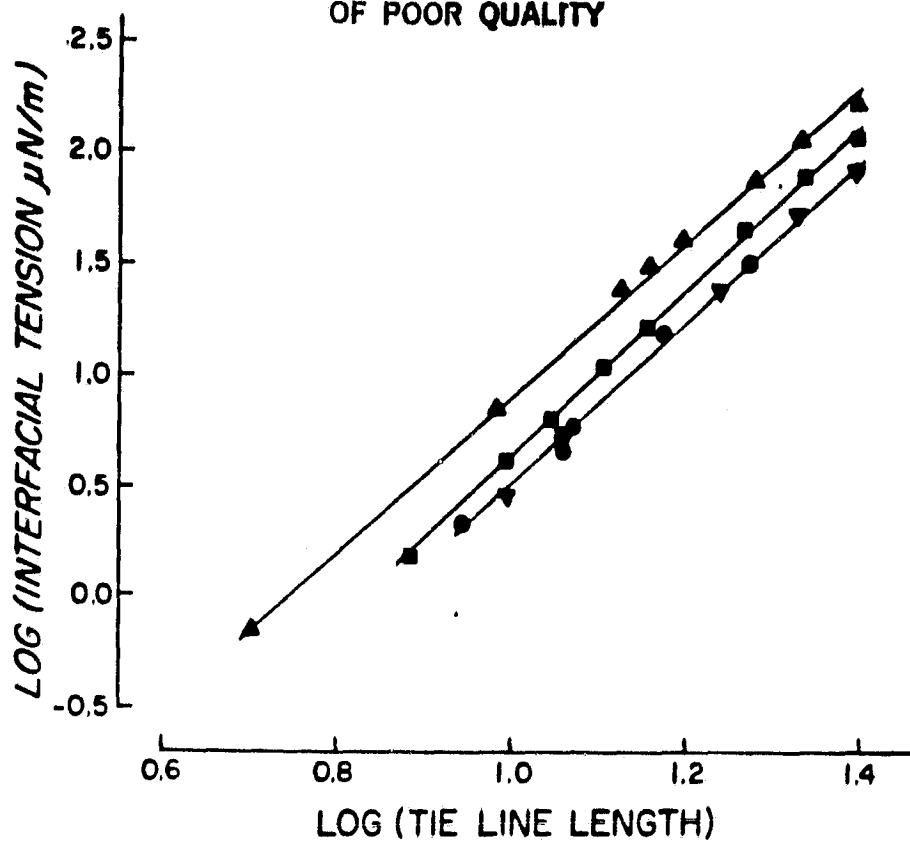
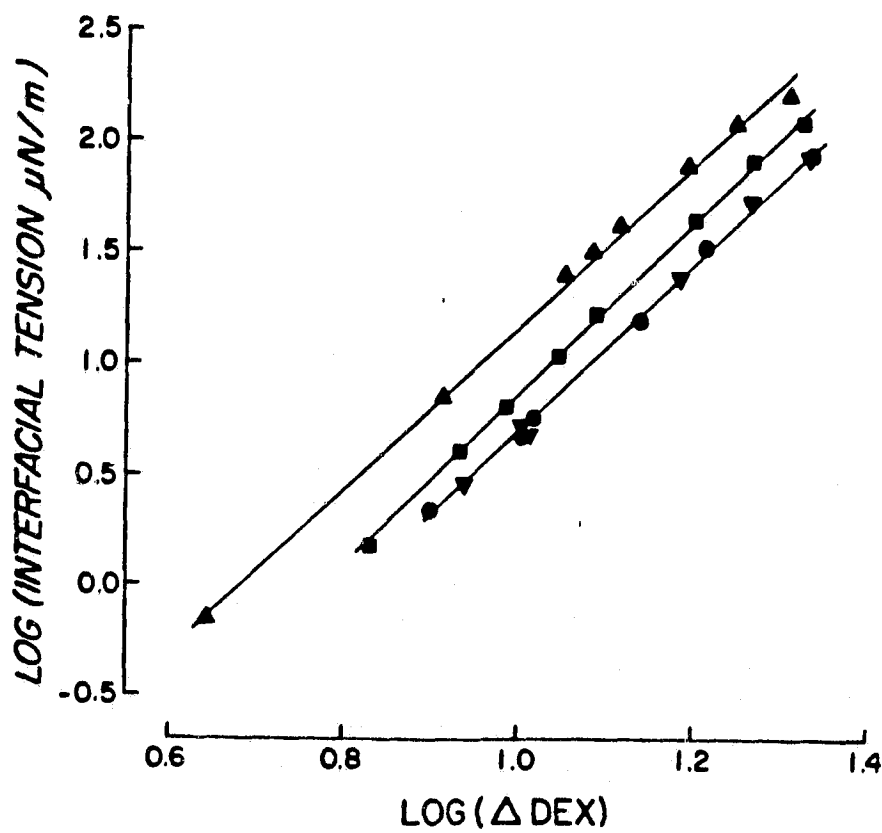


Fig. 4



ELECTROSTATIC AND ELECTROKINETIC POTENTIALS  
IN TWO POLYMER AQUEOUS PHASE SYSTEMS

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Running Title: Potentials in Two Polymer Phase Systems

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ABSTRACT

Two polymer aqueous phase systems have proven useful as partition media for biological cells, the partition coefficients obtained being dependent on cell surface properties. One such property appears to be the surface charge density of cells which prompted this detailed examination of electrostatic potential distributions in dextran/poly(ethylene glycol) (PEG) two phase systems. The partition coefficients of potassium sulfate and potassium chloride in the phase systems were measured and correlated with the Donnan potential difference between the phases, estimated via measurements with salt bridges to reversible electrodes. Thermodynamic predictions of the relationship between the two measured quantities were confirmed. The preference of sulfate for the dextran-rich bottom phase, which linearly increases with increasing PEG concentration, is due to the greater exclusion of sulfate by PEG than by dextran, as indicated by equilibrium dialysis of the polymer against different salt concentrations. Electrokinetic studies of droplets of one phase suspended in the other phase revealed relatively large electrophoretic droplet mobilities which increased linearly with the drop diameter and supralinearly with the sulfate concentration. The sign of the mobility depended on the phase the droplet originated from, but the implied surface charge sign was opposite to that anticipated from the difference in potential between the bulk phases. Assuming the mobility and zeta potential are of the same sign, the simplest potential profile which is consistent with these observations is one in which a dipole potential is present at the phase boundary oriented in such a way as to locally reverse the potential gradient. Levine's partial theory for the electrophoresis of systems of this type was tested and found to be consistent with our results. The correlations obtained suggest that the magnitude of the dipole potential is related to the anion partition coefficient in these systems.

## INTRODUCTION

Chemically distinct polymers frequently prove to be incompatible in a common solvent, phase separation resulting often at relatively low concentrations. Aqueous solutions of dextran (poly( $\alpha$ -1,6 glucose)) and poly(ethylene glycol) (PEG) undergo phase separation if more than a few percent of each are present. Those polymer solutions are non-toxic towards biological materials and, since dextran and PEG bear no ionogenic groups, they are readily buffered to physiological conditions. As Albertsson originally showed, these phase systems can act as excellent support media for partitioning of cells, organelles and macromolecules (1). With cells and other particulates, the interface between the bulk phases can accumulate material by adsorption, so that, effectively, three compartments are present among which particles can distribute. The partition behavior can be utilized both analytically and preparatively and, particularly when exploited using sequential partition steps via countercurrent distribution, the procedure has proven to be remarkably sensitive to a variety of properties of the distributed materials. A number of unique cell separations have been accomplished including isolation of lymphocyte subpopulations which can be identified by specific surface markers (2) and distribution of a murine lymphosarcoma cell line showing changes in partitioning behaviour with alterations in metastatic potential (3). Several recent reviews are available describing the general features of the partition procedure and its application in biological systems (4-6).

The experimental results accumulated to date clearly show that the partition behavior of cells in dextran/PEG phase systems is determined by properties of the cell membrane. Depending on the composition of the phase

system, the surface features to which partition can be made sensitive include cell surface charge (7,8), lipid and hydrophobic properties (9,10) as well as a set of as yet unidentified characteristics. In general, cell partition is affected by the concentrations and molecular weights of the two polymers, the interfacial tension of the liquid-liquid phase boundary, the degree to which the polymers interact with the cell surface and the type and concentration of the salts present. Many of these factors are mutually dependent, of course, and, in spite of significant effort in this area (1, 11-15), the details - and perhaps even the concepts (16) - of the relationship between phase system properties and partition are not yet understood.

In the present work, aspects of the basis for electrical effects in the partition process are examined. Johansson originally showed that some salts partition unequally in dextran/PEG phase systems (11). Reitherman et al. (12) subsequently demonstrated that, in the presence of phosphate, an electrostatic potential developed between the two phases, the presence and magnitude of which correlated with the appearance and degree of cell surface charge-associated partition. Johansson later correlated such potential differences with the partition behavior of the salts in the system (17). Specifically, it was shown that phosphates, citrate and sulfate all tended to partition to some degree in favor of the bottom, dextran-rich phase in dextran/PEG systems. We report here the results of detailed studies on ionic distributions in sulfate-containing systems and the consequences for the electrical properties of these solutions.

## MATERIALS AND METHODS

### Phase Systems

Phase systems were made up from concentrated stock solutions of dextran T500 (circa 20% w/w, Lot 5996,  $\bar{M}_w = 495,000$ ,  $\bar{M}_n = 190,000$  or Lot 3447,  $\bar{M}_w = 511,000$ ,  $\bar{M}_n = 191,600$ , Pharmacia, Uppsala, Sweden) and PEG 6000 (now called Carbowax 8000) (circa 40% w/w,  $\bar{M}_w \sim 8,000$ , Union Carbide, New York, NY). Dextran stock concentrations were measured polarimetrically (Circle Polarimeter, Carl Zeiss, Oberkochen, West Germany) using a specific rotation of  $[\alpha]_D^{25} = +199^\circ$  (1). PEG stock solutions were made up by weight. All phase systems were prepared from these stocks on a weight basis using a top loading balance because of the difficulty of conducting volumetric manipulations with the viscous polymer stock solutions. Dextran solution densities were measured pycnometrically and the results used to convert the volumetric concentrations measured polarimetrically to a weight basis. Polymer compositions of separated phases were determined by a combination of polarimetry and refractive index measurements (Abbe Refractometer, Bausch and Lomb, Rochester, NY), the PEG concentrations being determined by subtracting the refractive index increments, due to the known dextran and salt concentrations, from the measured values.

The tie line length (TLL) was calculated from

$$TLL^2 = (D_1 - D_2)^2 + (P_1 - P_2)^2 \quad [1]$$

where  $D_1$ ,  $D_2$  are the concentrations of Dextran in each phase  
and  $P_1$ ,  $P_2$  are the concentrations of PEG in each phase.

Concentrated salt stocks (0.4 M  $K_2SO_4$ , 1.0 M KCl, 1.0 M  $KNO_3$ ), where necessary supplemented with dry salt, were added by weight to give the desired compositions. All salt concentrations in the phase systems are given in moles of salt per kg solutions (moles/kg). All salts were reagent grade. Phase separation was allowed to take place either by leaving each system overnight in a separatory funnel at 25°C or by centrifugation at 1,200 xg for 10 min. Viscosities of separated phases were measured with an Ostwald capillary viscometer.

### Salt Partition

Partition coefficients of  $K_2SO_4$  and  $KNO_3$  in the phase systems were determined radiochemically utilizing trace amounts of  $^{35}SO_4^{2-}$  (carrier-free, New England Nuclear, Boston, MA) and  $^{42}K$  (1.12 mCi/mg, New England Nuclear), while KCl partition was measured via conductimetric titration as described earlier (19). For the  $SO_4^{2-}$  measurements phase systems (5 g) were made up with the required composition utilizing salt stocks containing 0.4 M  $K_2SO_4$  and 0.8  $\mu Ci/ml$   $^{35}SO_4^{2-}$  or 0.1 M  $K_2SO_4$  and 0.20  $\mu Ci/ml$   $^{35}SO_4^{2-}$ . Following centrifugation duplicate 0.4 ml aliquots of top and bottom phases were diluted to 2.0 ml with distilled water and 1 ml samples counted in a Beckman scintillation counter (Fullerton, CA) in the  $^{14}C$  channel. The scintillation cocktail used (10 ml per vial) consisted of 0.4 g PPO (Calbiochem, La Jolla, CA) plus 10 mg dimethyl POPOP (Packard, Downers Grove, IL) in 200 ml toluene to which was added 100 ml Triton X-100 (Sigma, Saint Louis, MO). Absolute counting efficiencies were not determined, but the ratio of efficiencies of the top:bottom phase counts was determined. It was equal to the ratio of counts measured for separated top and bottom phases made up without isotope, to which equal amounts of  $^{35}SO_4^{2-}$  were subsequently added. This ratio was used to correct the measured ratio of activity in the two phases to give the true partition coefficient.  $KNO_3$  partition coefficients

were measured directly on 0.4 ml aliquots of equilibrated top and bottom phases containing of the order of 50,000 cpm of  $^{42}\text{K}$  via gamma counting in a Nuclear Chicago well counter (Searle Analytic, Des Plaines, IL).

Equilibrium dialysis determinants of  $\text{SO}_4^{2-}$  distribution between dextran or PEG and polymer-free salt solutions were carried out utilizing Spectrapor dialysis tubing (Spectrum Medical Industries, Los Angeles, CA) with a 2,000 dalton cut-off, mechanically reinforced with a double casing of nylon mosquito netting. Aliquots (2-3 ml) of  $\approx 60\%$  PEG or  $\approx 50\%$  dextran were first dialyzed against two changes of deionized water for 24 hr to remove any dialyzable polymer, then equilibrated for 72 hr against the appropriate salt solutions containing  $\approx 1 \times 10^6$  cpm  $^{35}\text{SO}_4^{2-}$  in approximately 225 ml in the PEG experiments and  $\approx 6 \times 10^6$  cpm in approximately 125 ml in the dextran measurements. The initial salt concentration was such as to reach a final nominal concentration in the bathing medium of the values indicated in Results. The  $^{35}\text{SO}_4^{2-}$  concentration inside and outside the dialysis sacs were then determined with a Philips PW 4700 scintillation counter using 0.5 ml aliquots of phase system diluted with 1.0 ml deionized water to which 11 ml of Atomlight (New England Nuclear) was added. Counting efficiencies in these experiments were essentially independent of polymer concentration, so were not routinely measured. All equilibrium dialysis experiments were carried out at room temperature ( $21 \pm 1^\circ\text{C}$ ).

#### Bulk Phase Potential Measurements

Electrostatic potentials between the top and bottom phases were measured two ways. In the first, following Reitherman et al. (12), salt bridges were pulled from pasteur pipettes, filled with 2% agar and 3 M KCl, Ag-AgCl electrodes inserted and connected to either a Vibron 33B-2 electrometer or a



Hewlett Packard Model 3465A Digital Volt meter, both of which had input resistances  $>10^{10} \Omega$ . In the second method, capillary electrodes were pulled to an internal tip diameter of  $\sim 30 \mu\text{m}$  utilizing a David Knopf Instruments vertical pipette puller and filled with 1.0 M KCl, producing electrode resistances of  $\sim 1.5 \text{ M}\Omega$ . The entire measurement assembly was enclosed in a Faraday cage. Potential differences were measured as the differences in steady potential recorded when one electrode was moved through the interface from one phase to the other, the second electrode remaining in one phase (usually the top phase). At least 10 such readings were averaged to give the values quoted. The results obtained were independent of phase volume. All phase systems were allowed to settle overnight at  $25.0 \pm 0.1^\circ\text{C}$  before measurements were made.

#### Electrophoretic Mobility Measurements

Electrophoretic mobilities of drops of one phase suspended in the other were determined at  $25.0 \pm 0.1^\circ\text{C}$  in a microelectrophoresis apparatus equipped with a cylindrical chamber and Ag/AgCl electrodes, as described for biological cells by Seaman (18). Drops were generated by adding 10  $\mu\text{l}$  of bottom phase to 5 ml of top phase, or 20  $\mu\text{l}$  of top phase to 5 ml of bottom phase and agitating gently. Phase drop diameters were measured in the electrophoresis chamber at the stationary level with the electric field off via a filar micrometer eyepiece (American Optical, Buffalo, NY), then the field turned on (typically 1-3 V/cm) and the migration of the droplets timed over a known distance.

## RESULTS

Figure 1 shows the potentials measured on the same set of  $K_2SO_4$  containing systems using either agar-containing salt bridges, or microcapillary salt bridges, both filled with 1 M KCl. Surprisingly the agar bridges gave a lower potential, which decreased with increasing sulphate concentration, while the values obtained with the microcapillaries were independent of concentration up to 30 mM sulphate. The microcapillary bridges were used for all further potential measurements.

The tie line length, potential and salt partition coefficients,  $K$ , for KCl and  $K_2SO_4$  containing systems are given in Tables 1,2 and 3 for a variety of polymer compositions and salt concentrations. The single ion activity coefficients ( $f$ ) of potassium in each phase were calculated from  $K$ , the bulk salt concentration and the Debye Huckel expression (20)

$$\text{Log } f^{\circ} = \frac{-A I^{1/2}}{1 + Ba(I)^{1/2}} \quad [2]$$

with  $A = 0.5115 \text{ mole}^{-1/2} \text{ l}^{1/2}$ ,  $B = 0.3291 \text{ \AA}^{-1} \text{ mole}^{-1/2} \text{ l}^{1/2}$ ,  $a = 3.11 \text{ \AA}$  for  $K_2SO_4$  systems,  $a = 4.25 \text{ \AA}$  for KCl systems.  $I$  is the ionic strength.

The  $K$  for KCl was constant, while there was a small decrease in both the potential and the tie line length with increasing KCl concentration (Table 1). By contrast, for 5% Dextran, 4% PEG systems containing more than 30 mM  $K_2SO_4$  there is an increase in the tie line and potential, and a corresponding decrease in  $K$  with increasing  $K_2SO_4$  concentration. (Table 2) The partition coefficient for  $KNO_3$  was close to 1.0 and was independent of salt concentration in all systems tested, as seen in Table 4. This table also contains values for the densities and viscosities of the phase systems studied

electrokinetically.

The  $K_2SO_4$  partition behavior in the phase systems is confirmed and clarified somewhat by the results of the equilibrium dialysis experiments summarized in Fig. 2 and Table 5. Figure 2 illustrates that  $K_2SO_4$  is quite strongly rejected from PEG solutions and that dextran also excludes  $SO_4^{2-}$ , although to a considerably lesser degree. The exclusion appears to increase linearly in magnitude with increasing polymer concentration in both cases. The  $SO_4^{2-}$  partition coefficient varied only slightly, if at all, as a function of  $K_2SO_4$  concentration, however, in apparent contrast to the results in Table 2. In fact the correlation coefficient for a plot of the slope of  $SO_4^{2-}$  partition coefficient as a function of polymer concentration (Table 5) with the total salt concentration is not significant at the 5% level for either PEG or dextran exclusion (plot not shown). The data in Table 6, provide the likely explanation as they show that the polymer concentrations in the two phases and hence the tie line lengths differ depending on the  $SO_4^{2-}$  concentration, increasing sulfate being associated both with greater ionic exclusion and greater differences in PEG concentration between the upper and lower phases. The data in Table 6 were used to calculate the sulfate partition coefficient as a function of the difference in PEG concentration between the phases; these data are also included in Fig. 2, the line being indistinguishable from the dialysis results for pure PEG solutions.

When the electrokinetic properties of drops of one phase in the other were examined, it became apparent that the electrophoretic mobility depended strongly on the drop diameter, regardless of which phase was examined. The dependence on drop size is shown for a 5% dextran, 4% PEG (5/4) system made up in 0.2 M  $K_2SO_4$  in Fig. 3; clearly the magnitude of the mobility increases

linearly with drop diameter over the size range examined. It was also found that this relationship was independent of the electric field strength between 1 and 3 V/cm. For a standard drop size of nominally 7  $\mu\text{m}$  diameter the dependence of the top and bottom phase drop mobilities on salt composition is shown for two different 5/4 systems in Figs. 4 and 5. Figure 4 illustrates that in systems containing only  $\text{K}_2\text{SO}_4$  the mobilities of the top and bottom phase drops are of opposite sign, the drop mobility being positive when the bottom, dextran-rich phase is dispersed in the PEG-rich phase and the mobility being negative when the PEG-rich phase forms the drops suspended in the dextran-rich phase. The mobilities of both types of drops increase in magnitude with increasing sulfate concentration and ionic strength, the increase being supralinear above about 0.2 moles/kg  $\text{K}_2\text{SO}_4$ , but symmetrical within a scaling factor for both phases. When the 5/4 systems are made up with varying ratios of  $\text{KNO}_3$  and  $\text{K}_2\text{SO}_4$  at a constant ionic strength of approximately 0.3 M, again the two curves are symmetrical but change sign as the  $\text{SO}_4^{2-}$  is increased from 0 to 0.1 moles/kg and the  $\text{KNO}_3$  reduced from 0.3 moles/kg to 0, the curves crossing at zero mobility (Fig. 5).

# DISCUSSION

Although electrodes filled with agar to reduce KCl leakage have traditionally been used for electrostatic potential measurements of the type made here (12), the values obtained with the microelectrodes containing no agar provided data which were more consistent with the measured salt partitions and the expected potentials based on the thermodynamic analysis given below. We hypothesize that the differences illustrated in Figure 1 are due to a potential introduced at the agar-phase boundary at the electrode tips, which changes as a function of system composition. Use of high resistance microcapillary electrodes seemed to avoid most of the complications of the measurements.

Absolute potential measurements cannot be made in a strict thermodynamic sense, due to the unknown effect of liquid junction potentials at the tips of the salt bridges. It is therefore necessary to demonstrate that the measured potentials are consistent with the observed partition coefficients of KCl and  $K_2SO_4$ . One approach, using differences in potentials, can be made as follows:

At equilibrium the chemical potentials of an ionic species partitioning between two phases are equal. Hence for the common ion,  $K^+$ , in KCl and  $K_2SO_4$  containing systems.

$$\begin{aligned} \mu_{CT}^0 + RT \ln f_{CT} C_{CT} + F\psi_{CT} &= \mu_{CB}^0 + RT \ln f_{CB} C_{CB} + F\psi_{CB} \\ \mu_{ST}^0 + RT \ln f_{ST} C_{ST} + F\psi_{ST} &= \mu_{SB}^0 + RT \ln f_{SB} C_{SB} + F\psi_{SB} \end{aligned} \quad [3]$$

where the subscripts T and B refer to the values in the upper and lower phases respectively, and C and S refer to systems containing KCl or  $K_2SO_4$  respectively. All quantities refer to the potassium ion.

$\mu^0$  is the standard state chemical potential

$f$  is the activity coefficient

$c$  is the concentration

$\psi$  is the electrostatic potential

$F$  is the Faraday constant

Solving for the potential difference between the phases,  $\Delta\psi = \psi_B - \psi_T$ ,

$$\Delta\psi_C = RT \ln K_C + RT \ln r_C + \Delta\mu_C^0 \quad [4]$$

where

$$K_C = c_{CT}/c_{CB}, \quad r_C = f_{CT}/f_{CB} \quad \text{and} \quad \Delta\mu_C^0 = \mu_{CB}^0 - \mu_{CT}^0 \quad [5]$$

Similarly for  $K_2SO_4$ -containing systems, one obtains

$$\Delta\psi_S = RT \ln K_S + RT \ln r_S + \Delta\mu_S^0$$

Subtracting equations 4 and 5 gives:

$$\Delta\psi_S - \Delta\psi_C = RT \ln K_S/K_C + RT \ln r_S/r_C + (\Delta\mu_S^0 - \Delta\mu_C^0) \quad [6]$$

By taking the difference between potentials in systems with the same nominal polymer concentrations the effect of the liquid junction potentials should cancel. This approximation is most valid when the polymer compositions of the two systems being compared are most similar, ie. at low salt concentrations. Similarly, although the last term on the right hand side is not measurable, it should vanish under the same conditions. Equation 6, without the right hand term, was used to calculate the expected potentials in  $K_2SO_4$  systems containing 5% Dextran, 4% PEG systems, Table 2. There is reasonable agreement between the predicted and observed potentials at low sulfate concentration.

However as the concentration increases the phase composition alters, as indicated by the change in tie line length, and the discrepancy becomes progressively larger. Such behavior is expected based on the considerations given above in the derivation of Equation 6. In the systems on which Table 3 is based the polymer concentrations were adjusted to give tie line lengths closer to those of the KCl-containing systems, and better agreement of the predicted and observed potentials is obtained at all sulfate concentrations. This demonstrates that the increase in sulfate concentration itself is not the cause of the discrepancies in Table 2, but that it is due to the effect of the salt on the polymer composition. Hence, it would appear that the experimental measurements satisfactorily confirm the thermodynamic predictions and therefore that differences in potentials in different phase systems measured as discussed herein can be used to describe electrostatic effects on partition behavior in these systems.

It is evident from the results presented in Fig. 2 and Tables 5 and 6 that, although neither dextran nor PEG bears a detectable number of ionogenic groups, both polymers can affect the distribution of  $\text{SO}_4^{2-}$  ions in phase separated solutions or in equilibrium dialysis experiments.  $\text{NO}_3^-$  ions, on the other hand, distribute approximately evenly between the phases in spite of some similarity in hydrogen bonding capability and structure.  $\text{Cl}^-$  ions partition weakly in favor of the bottom phase in all systems. Both PEG and dextran exclude  $\text{SO}_4^{2-}$  to a degree which is linearly dependent on polymer concentration. PEG is much more effective in this regard, the exclusion being more than ten fold greater per gram of polymer compared to dextran. We have obtained qualitatively similar results for phosphate distribution in comparable systems (19).

Since the equilibrium dialysis experiments show that the exclusion is not significantly dependent on the  $K_2SO_4$  concentration between 0.1 M and 0.4 M, the apparent ionic strength dependence seen in Table 2 is unlikely to be due to a direct effect of this type. The effect instead seems to originate from the influence of the increasing  $K_2SO_4$  concentration on the polymer compositions of the equilibrium phases. The data in Table 6 show that in the presence of  $SO_4^{2-}$ , which is itself capable of inducing phase separation in pure PEG solutions at high concentrations (1), the polymer equilibria shift in response to the ionic exclusion so that successively larger differences in PEG concentration between the phases occur the higher the  $SO_4^{2-}$  concentration. Concomitantly, the difference in dextran concentration between the phases decreases somewhat with increasing salt (except for phase systems near the critical polymer concentrations, below which phase separation will not occur). Both these changes are in the direction which will increase the partition of  $SO_4^{2-}$  into the bottom, dextran-rich phase due to its exclusion by the PEG-rich upper phase as the salt concentration is increased. That the magnitude of the effect observed in the phase systems is consistent with the dialysis results obtained on pure polymer solutions is shown by the data in Fig. 2, in which the sulfate partition coefficient in a series of phase systems is plotted as a function of the difference in PEG concentrations between the phases. The points fall on the same straight line as defined by the values for pure PEG. Dextran tends to exclude  $SO_4^{2-}$  weakly, hence its predominance in the bottom phase does not have a detectable effect. The exclusion effects of the two polymers in the phase systems, therefore, appear to be very similar to their effects in pure solution.



The ion exclusion recorded in Table 2, which results in the partition of  $\text{SO}_4^{2-}$  out of the top phase into the bottom, would be expected to create a Donnan potential between the phases, with the  $\text{SO}_4^{2-}$ -accumulating bottom phase negative with respect to the top phase. The potential measurements shown in Fig. 1 are consistent with this expectation.

A second method by which to examine the potential distributions in these systems is to measure the electrokinetic properties of drops of one phase suspended in the other (21). The data plotted in Figs. 3, 4, and 5 characterize the present system in this regard. The results show considerable differences from the electrokinetic behavior of solid particles in that the mobilities depend linearly on the particle diameter, show very large values, particularly considering the viscosities present, and increase strongly with increasing ionic strength. None of these characteristics are exhibited by solid particles which derive their charge via ion adsorption or the dissociation of surface ionogenic groups. Presumably, therefore, the properties observed are due to a combination of the fluid nature of the body undergoing electrophoresis, which allows internal circulation to occur within the drop, and the charging mechanism at the phase boundary, about which nothing is known.

By measuring the electrophoretic mobilities of drops of each phase suspended in the other, in principle the potential between the interface and each of the two bulk phases can be estimated as the zeta potential of each of the two types of drops. Unfortunately, however, there is no complete theory available with which to interpret the measured electrophoretic mobilities in order to derive the required zeta potentials. Theories for the electrokinetic properties of mercury drops with perfectly polarizable interfaces have been

developed (22), but they clearly do not apply to the free liquid interface present in phase-separated polymer systems. Levine has begun an analysis of this problem utilizing the observations presented here and elsewhere (23). He is developing a theory for the electrophoresis of a liquid drop bearing a layer of point dipoles at the interface, the drop maintaining a potential difference between the exterior and interior via ionic partitions of the type just discussed (24). Assuming phase systems in which the dielectric constant, conductivity and ionic strength are equal in the two phases, a partial expression can be obtained for the mobility  $U$ , namely:

$$U \approx \frac{\epsilon_0 \kappa_0 \zeta_0 a^2}{2\pi(2\eta_0 + 3\eta_i)} \left( \frac{A_0}{a^3} \right)$$

where  $\epsilon_0$  = dielectric constant of suspending medium

$\kappa_0$  = Debye Hückel parameter for suspending medium (20)

$\zeta_0$  = zeta potential of drop

$a$  = drop radius

$\eta_0$  = viscosity of suspending medium

$\eta_i$  = viscosity of phase in drop

$A_0$  = undetermined function with dimensions of volume

The zeta potential is related to the dipole and bulk phase potential differences by (assuming a drop of top phase):

$$\zeta_0 = 1/2(\Delta V + \Delta\psi)$$

where  $\Delta V$  = dipole potential at the interface

$\Delta\psi$  = Donnan potential difference between the top and bottom phases,  
as defined previously

Although we do not know the magnitude of  $\Delta V$ , the zeta potential

ought to be linear in  $\Delta\psi$ . Since  $\kappa_0$  is proportional to the square root of the  $K_2SO_4$  concentration, a partial test of equation [4] should be provided by a plot of  $U(2\eta_0 + 3\eta_1)[K_2SO_4]^{-1/2}$  vs  $\Delta\psi$ , where  $[K_2SO_4]$  is the  $K_2SO_4$  concentration for drops of constant size. Figure 6 shows the data in this form. It is seen that a linear relationship is in fact obtained both for top phase and bottom phase drops in the presence of different sulfate concentrations and that the slopes of the two lines are equal, except for the point at the lowest salt concentration. This suggests that Levine's theory correctly predicts the effects of viscosity, and perhaps ionic strength, in these systems. The results also suggest that the magnitude of the zeta potential is related to the log of the salt partition coefficient, since the bulk phase potential difference is directly determined by that parameter (equation [4]). However, it is seen from a comparison of Figs. 1 and 4 that the sign of the zeta potential, assumed to be equal to the sign of the measured electrophoretic mobility, is in fact opposite to that expected from the salt partition and  $\Delta\psi$  measurements. That is, drops of the bottom, dextran-rich phase, which accumulate  $SO_4^{2-}$  due to the exclusion of these ions by PEG and whose bulk phase is negative with respect to the suspending medium, exhibit a positive electrophoretic mobility, and vice versa for top phase drops.

The situation is illustrated in Figs. 7A and 7B where the potential profile anticipated from the equilibrium measurements (Fig. 7A) is contrasted with the simplest model which accounts for the observed behavior, assuming the mobility and zeta potential are of the same sign (Fig. 7B). It is evident that an electrical dipole must be present at the phase boundary, the dipole potential of which is not known but could be estimated if a complete theory

for the zeta potential were available.

The other feature evident from Fig. 7B is that the position of the shear plane cannot be the same for the two orientations of the interface when top and bottom phase drops are measured. There is no single point on the potential profile in Fig. 7B that will provide zeta potentials of the observed signs, given the sign of  $\Delta\psi$ . Hence the shear plane must shift in the radial direction, by approximately the projected length of the dipole moment, or more, when the curvature of the interface is reversed and the two different phases are made to undergo electrophoresis.

The potential differences measured or implied by the data presented here are, in principle, those which will influence the partitioning of exogenous charged materials introduced into the two phase systems. The bulk phase potential differences measured, shown here to be good approximations to the thermodynamic potentials, have been found to correlate well with the partition behaviour of charged particles (12). The zeta potentials, however, appear to be of opposite sign to those expected from the  $\Delta\psi$  measurements; hence their role in affecting partition behavior, particularly particle adsorption at the interface, is not clear at present. Without being able to calculate the magnitudes of the zeta and dipole potentials it is not possible to predict their effects. Particle partition data suggest that such effects may not be large, however.

ACKNOWLEDGEMENTS

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Table 1. KCl partition coefficients (K) and potential differences between the phases of 5% dextran T500, 4% PEG 6000 systems containing different concentrations of KCl. Quoted error is typical.

[KCL] (moles/ kg)	Tie Line Length (% w/w)	K	Molar Activity Coefficients			$\Delta\psi(\text{mV})$
			Top	Bottom	Ratio	
0.01	10.52	$0.974 \pm 0.018$	0.895	0.894	1.001	$0.4 \pm 0.03$
0.03	10.44	0.957	0.835	0.833	1.002	0.10
0.1	10.25	0.965	0.762	0.759	1.004	0.079
0.2	10.04	0.948	0.709	0.705	1.006	0.066
0.3	10.09	0.960	0.643	0.639	1.006	0.052
0.4	9.94	0.956	0.616	0.612	1.007	0.050



Table 2. Data for 5% dextran T500/4% PEG 6000 systems containing various concentrations for  $K_2SO_4$ .

[ $K_2SO_4$ ] (moles/ kg)	Tie Line Length (% w/w)	K	Molal Activity Coefficients		Measured $\Delta\psi$ (mV)	Predicted <sup>a</sup> $\Delta\psi_p$ (mV)	$\Delta\psi_p - \Delta\psi$ (mV)
			Top	Bottom			
0.001	n.d	0.888	0.940	0.937	2.27	2.00	-0.27
0.003	n.d	0.883	0.932	0.896	2.25	2.04	-0.21
0.01	n.d	0.880	0.839	0.831	2.25	1.82	-0.43
0.03	10.6 $\pm$ 0.2	0.875 $\pm$ 0.01	0.761	0.750	2.21 $\pm$ 0.05	2.54 $\pm$ 0.5	0.33
0.1	11.1	0.841	0.656	0.640	2.30	3.30	1.00
0.2	11.2	0.815	0.593	0.575	2.38	3.96	1.58
0.3	11.5	0.790	0.556	0.536	2.52	4.61	2.09
0.4	12.0	0.760	0.531	0.508	2.92	5.41	2.49

<sup>a</sup> In Tables 2 & 3 predicted potentials were calculated using pairs of  $KCl/K_2SO_4$  systems with the most similar tie line lengths. Quoted errors are typical and represent one standard deviation obtained from repeated measurements ( $\geq 3$ ) on the same phase system, or on duplicate phase system.

Table 3. Data for systems containing various concentrations of  $K_2SO_4$  and polymers

Dextran concentration (% w/w)	PEG concentration (% w/w)	$[K_2SO_4]$ (moles/ kg)	Tie Line Length(% w/w)	Salt Partition Coefficient	Molal Activity Coefficients Top Bottom	Ratio	Measured $\Delta\psi$ (mV)	Predicted $\Delta\psi_p$ (mV)	$\Delta\psi_p - \Delta\psi$ (mV)
5.00	4.00	0.030	10.6 $\pm$ 0.2	0.875 $\pm$ 0.01	0.761 0.750	1.015	2.21 $\pm$ 0.05	2.45 $\pm$ 0.5	0.33
4.85	3.88	0.10	10.5	0.856	0.660 0.642	1.029	1.82	2.75	0.93
4.63	3.70	0.20	10.0	0.881	0.592 0.581	1.020	1.42	1.60	0.17
4.27	3.42	0.30	9.25	0.910	0.554 0.546	1.014	1.14	0.91	-0.23
4.44	3.56	0.40	9.85	0.880	0.530 0.520	1.020	1.47	1.84	0.37

<sup>a</sup> In Tables 2 & 3 predicted potentials were calculated using pairs of  $KCl/K_2SO_4$  systems with the most similar tie line lengths.

Quoted errors are typical and represent are standard deviation obtained in repeated measurements ( $\geq 3$ ) on the same phase system, or on duplicate phase system.

Table 4.  $\text{KNO}_3$  partition coefficients and physical properties of 5% w/w dextran Lot #5996, 4% PEG phase systems,  $T = 25.0 \pm 0.1^\circ\text{C}$ .  $[\text{K}_2\text{SO}_4]$  = average  $\text{K}_2\text{SO}_4$  concentration in system, M/kg;  $[\text{KNO}_3]$  = average  $\text{KNO}_3$  concentration in system, moles/kg;  $K_{\text{NO}_3} = \text{NO}_3^-$  - partition coefficient;

$\eta$  = viscosity of separated phase,  $\text{dyne s cm}^{-2} \times 10^2$ ;  $\rho$  = density of separated phase,  $\text{g/cm}^3$ ; errors indicated are  $\pm 1$  standard deviation.

$[\text{K}_2\text{SO}_4]$	$[\text{KNO}_3]$	$K_{\text{NO}_3}$	Top		Bottom	
			$\eta$	$\rho$	$\eta$	$\rho$
-	0.01	$1.02 \pm 0.03$	3.29	1.012	27.01	1.042
-	0.40	$1.00 \pm 0.02$	3.29	1.036	25.06	1.065
0.01	-	-	3.29	1.013	26.47	1.043
0.02	-	-	3.31	1.014	26.87	1.044
0.05	-	-	3.26	1.018	26.67	1.048
0.10	-	-	3.21	1.024	28.43	1.056
0.20	-	-	3.19	1.037	30.39	1.071
0.36	-	-	3.69	1.061	28.31	1.101
-	0.30	$1.00 \pm 0.02$	3.27	1.030	25.89	1.057
0.02	0.24	1.03	3.23	1.030	27.90	1.063
0.05	0.15	1.05	3.16	1.025	28.26	1.059
0.075	0.075	1.10	3.16	1.025	28.65	1.058
-	-	-	3.30	1.012	25.90	1.042

Table 5. Linear regression analysis of equilibrium dialysis experiments measuring the partition coefficient of  $K_2SO_4$  as a function of PEG or dextran concentration at different average  $K_2SO_4$  concentrations  $[K_2SO_4]$ ,  $T = 21.0 \pm 0.5^\circ C$ .  $K_{SO_4} = K_2SO_4$  concentration ratio, inside/outside bag, corrected for volume occupied by polymer;  $[Polymer] =$  equilibrium polymer concentration inside bag, (% w/w).

Parameters of regression equation:

$$K_{SO_4} = \text{SLOPE} \times [Polymer] + \text{INT}$$

[Polymer]	$[K_2SO_4]$ (moles/kg)	SLOPE	INT
PEG	0.01	-0.0295	0.995
	0.1	-0.0318	0.992
	0.4	-0.0350	0.975
Dextran Lot #3447	0.01 to 0.4	-0.0023	1.012

Table 6.  $K_2SO_4$  partition coefficients and phase system compositions in dextran-PEG two phase systems,  $T = 21.0 \pm 0.5^\circ C$ . Total system composition in first column indicated X/Y/Z, where X = concentration of dextran Lot #3447, % w/w; Y = concentration of PEG, % w/w; Z = concentration of  $K_2SO_4$ , M/kg;  $K_{SO_4}$  = concentration ratio of  $K_2SO_4$ , top/bottom phase, corrected for volumes occupied for volumes occupied by polymers; [PEG] = PEG concentration, (% w/w); [DEX] = dextran concentration, % w/w; TLL = tie line length, defined in Materials and Methods.

Phase System Composition	$K_{SO_4}$	Top		Bottom		TLL
		[DEX]	[PEG]	[DEX]	[PEG]	
4/4/0.1	0.893	0.73	5.31	8.68	1.39	8.86
4/4/0.4	0.822	0.05	6.56	8.33	1.01	9.97
6/4/0.1	0.827	0.27	6.47	11.69	0.86	12.72
6/4/0.4	0.768	0.08	8.13	10.43	0.71	12.73
7/4/0.1	0.819	0.20	7.15	13.02	0.66	14.37
7/4/0.4	0.741	0.08	8.89	11.42	0.73	13.97

# FIGURE LEGENDS

Figure 1: Electrostatic potential difference between the two bulk phases of a 5% dextran, 4% PEG two phase system containing the average  $K_2SO_4$  concentrations indicated; top phase positive with respect to bottom phase;  $T = 25.0 \pm 0.1^\circ C$ ; (●) electrodes filled with 2% agar, 1M KCl; (o) microelectrodes filled with 1M KCl.

Figure 2: Partition coefficients of  $K_2SO_4$  in equilibrium dialysis experiments involving single polymer solutions and in the phase systems described in Table 3 as a function of PEG concentration (lower abscissa), dextran concentration (upper abscissa) or the difference in PEG concentration,  $\Delta PEG$ , between the phases (lower abscissa),  $T = 21 \pm 1^\circ C$ . (▲) sulfate distribution (concentration inside/outside bag) between dextran Lot #3447 solutions and simple salt solutions via equilibrium dialysis; (o) sulfate distribution between PEG solutions and simple salt solutions via equilibrium dialysis; (o) sulfate partition coefficients (concentrations in top/bottom) in two phase systems.

Figure 3: Absolute value of the electrophoretic mobility of drops of one phase suspended in the other as a function of drop diameter for a 5% w/w dextran Lot #5996, 4% w/w PEG, 0.2 M/kg  $K_2SO_4$  phase system;  $T = 25.0 \pm 0.1^\circ C$ ; (●) bottom phase drops suspended in top phase exhibiting positive mobilities; (■) top phase drops suspended in bottom phase exhibiting negative mobilities; left hand ordinate for bottom phase drops (B/T), right hand for top phase drops (T/B).

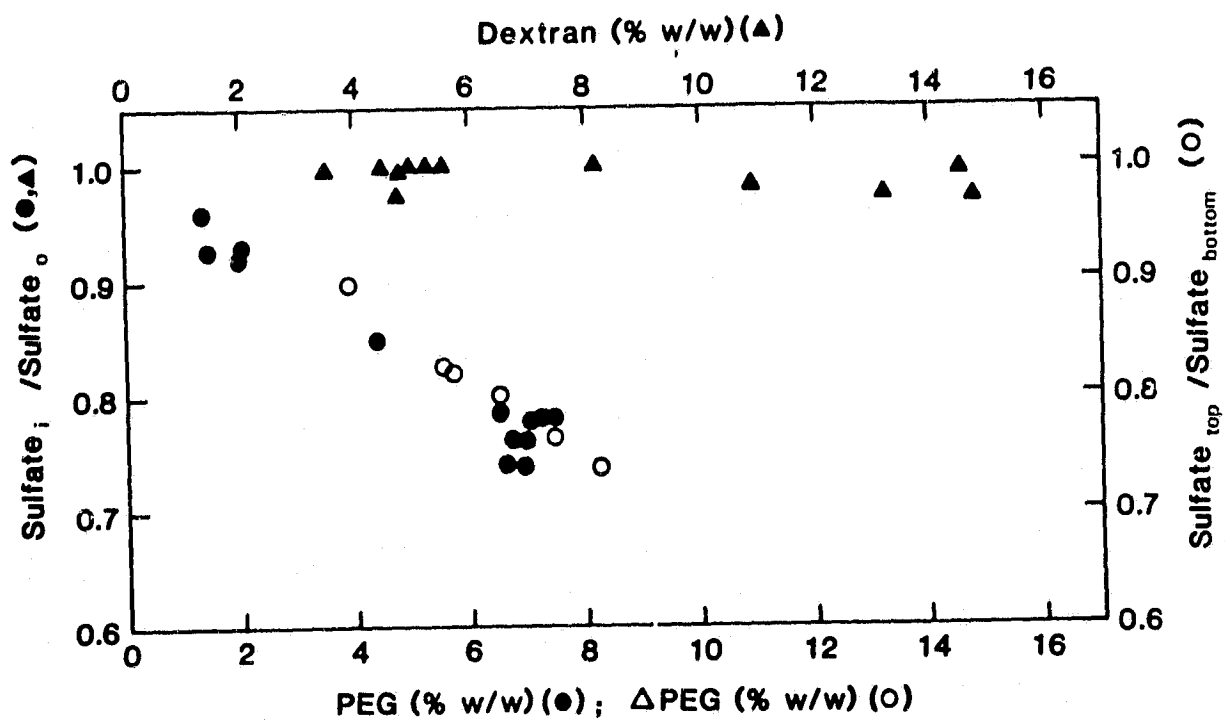
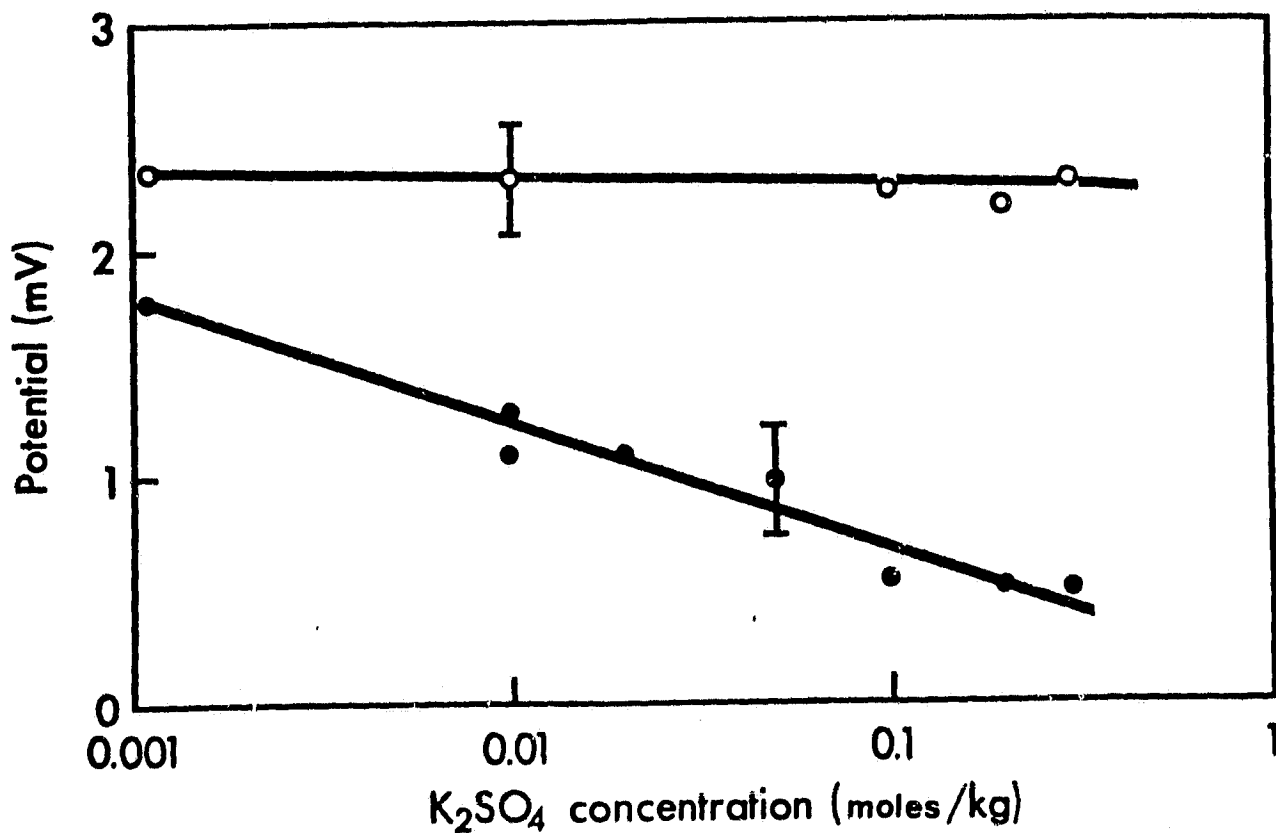
Figure 4: Electrophoretic mobility of drops of one phase suspended in the other as a function of the average concentration of  $K_2SO_4$  in 5% w/w

dextran Lot #5996, 4% w/w PEG phase system, average drop diameter =  $7.5 \pm 1.5$   $\mu\text{m}$ ,  $T = 25.0 \pm 0.1^\circ\text{C}$ .

Figure 5: Electrophoretic mobility of drops of one phase suspended in the other for a series of 5% w/w dextran Lot #5996, 4% w/w PEG phase systems containing the concentrations of  $\text{K}_2\text{SO}_4$  and  $\text{KNO}_3$  indicated; drop diameter =  $7.5 \pm 1.5$   $\mu\text{m}$ ;  $T = 25.0 \pm 0.1^\circ\text{C}$ .

Figure 6: A test of Levine's theory of phase drop electrophoresis. See text for explanation; ( $\Delta$ ) parameters for top phase drops; ( $\blacktriangle$ ) parameters for bottom phase drops; horizontal error bars calculated based on a single standard deviation of the measured potential differences; cumulative errors vertically were much less than those shown horizontally (values not shown).

Figures 7A and 7B: The anticipated electrostatic potential profile between the phases based on the sulfate partition and bulk phase potential measurements (7A) and the simplest potential profile consistent with both the electrokinetic and equilibrium measurements (7B); see text for discussion;  $\psi(x)$  = electrostatic potential profile;  $\Delta\psi$  = bulk phase potential difference;  $\zeta_{\text{B/T}}$  = zeta potential of bottom phase drops suspended in top phase;  $\zeta_{\text{T/B}}$  = zeta potential of top phase drops suspended in bottom phase.





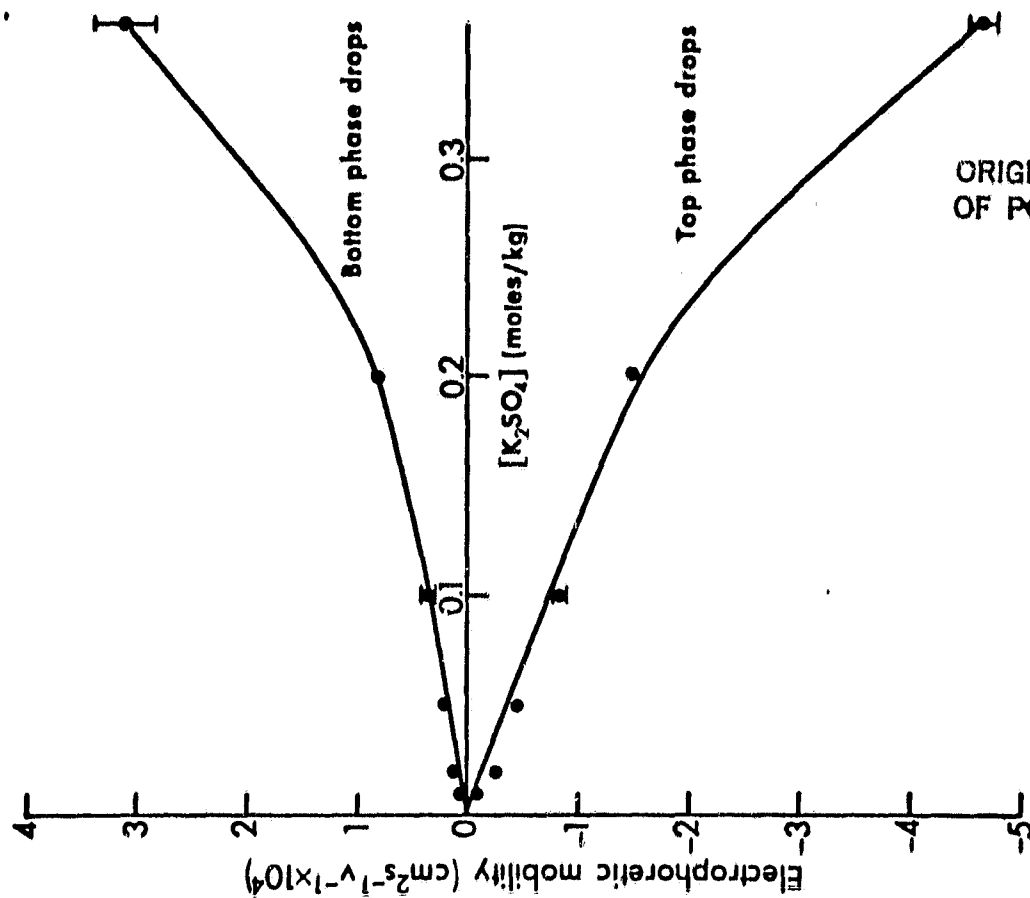


FIG. 4

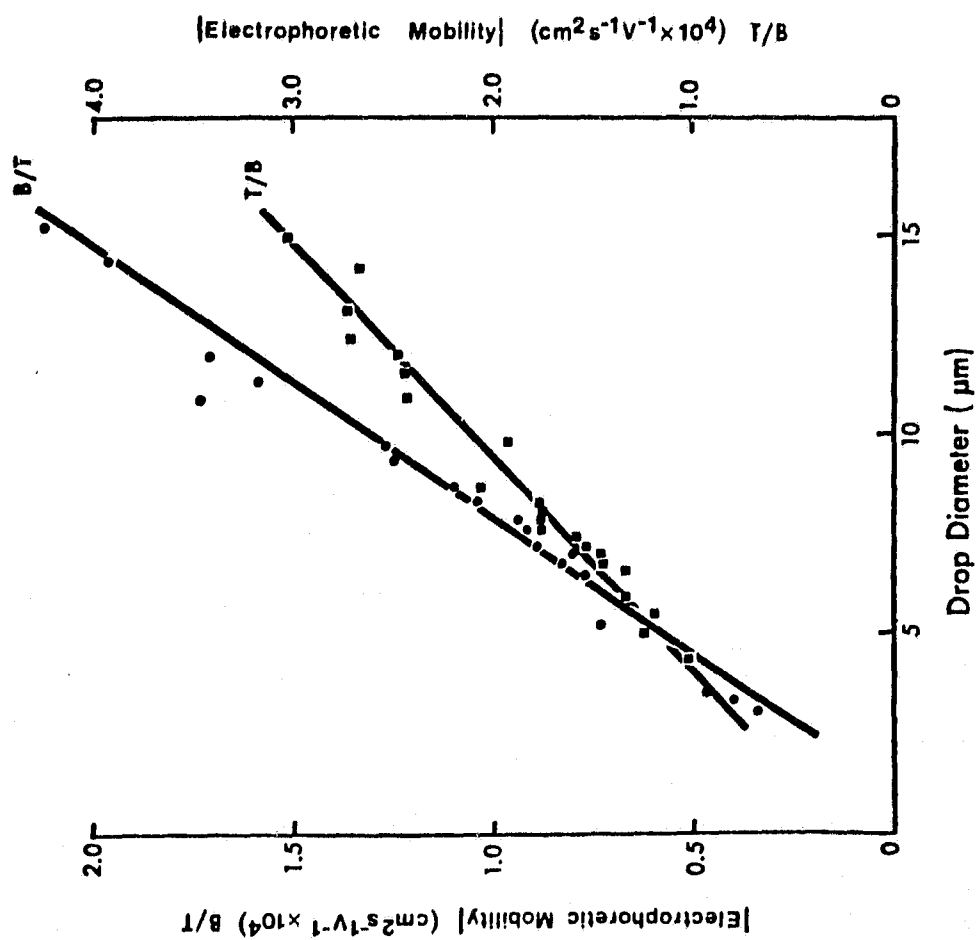
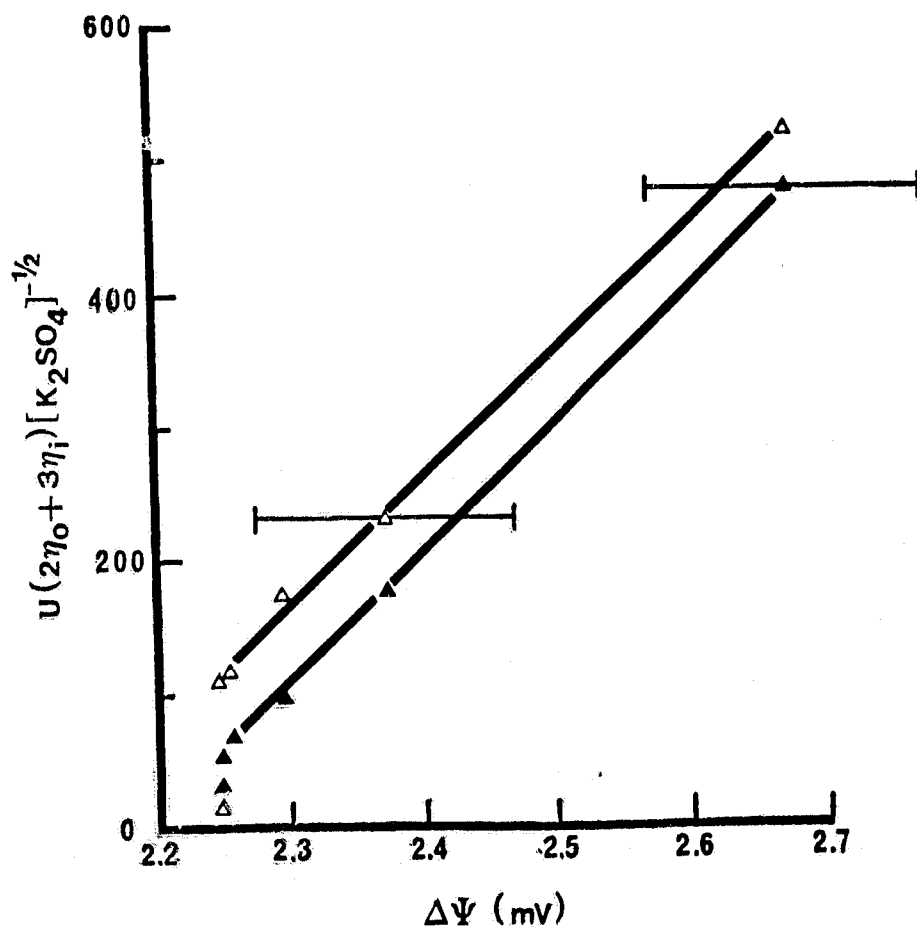
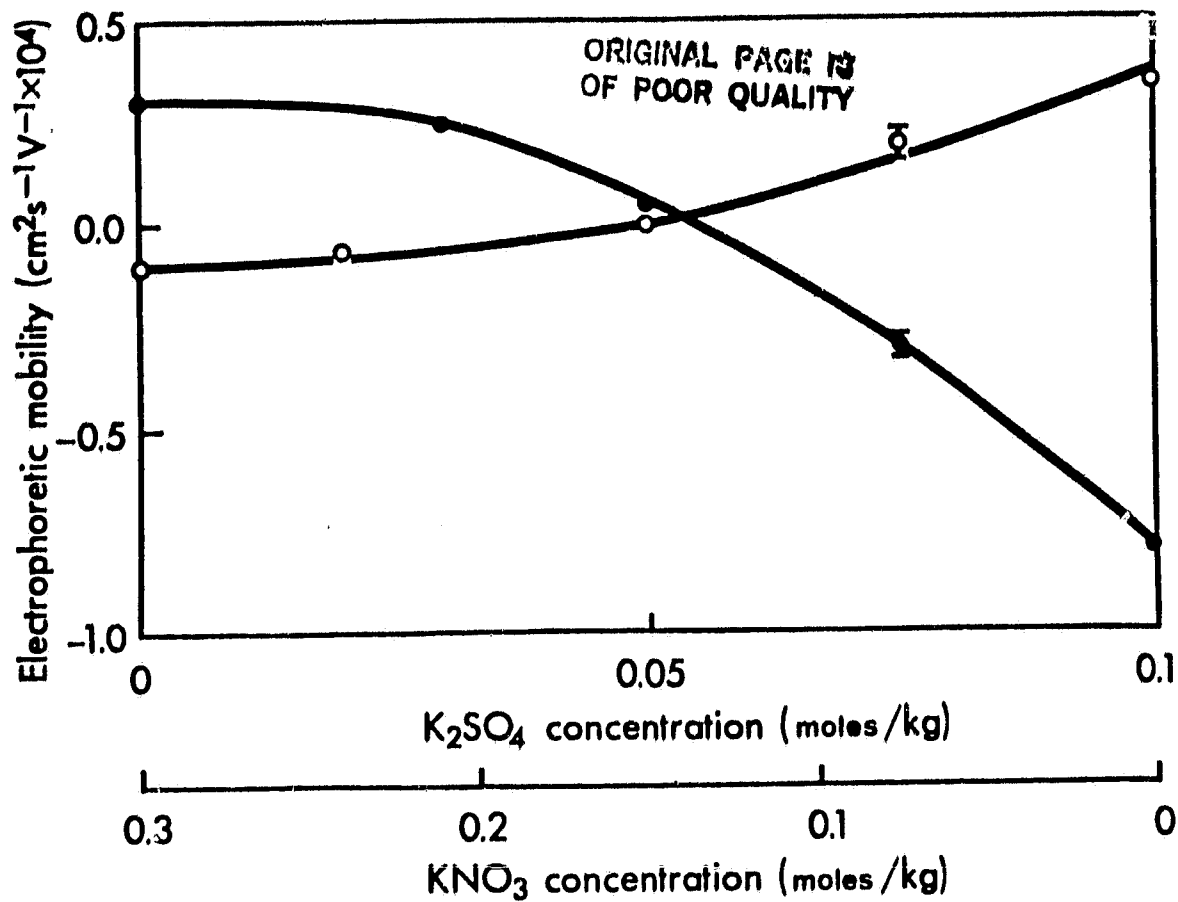


FIG. 3

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# Expected Potential Profile

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Figure 7A

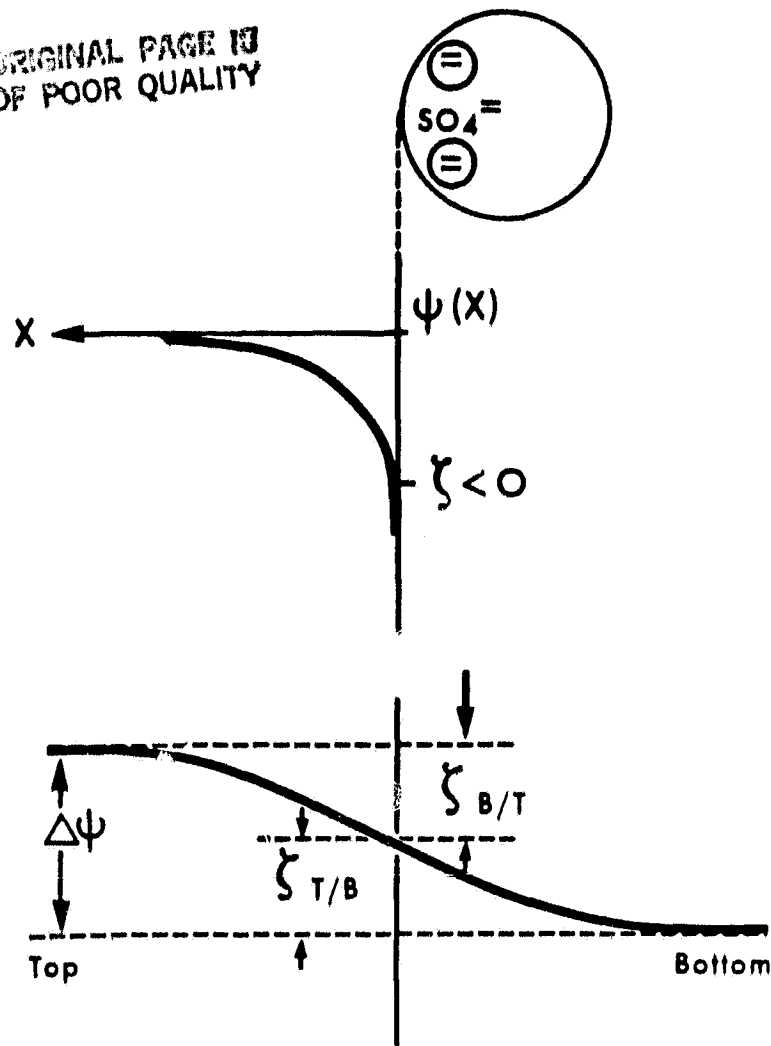
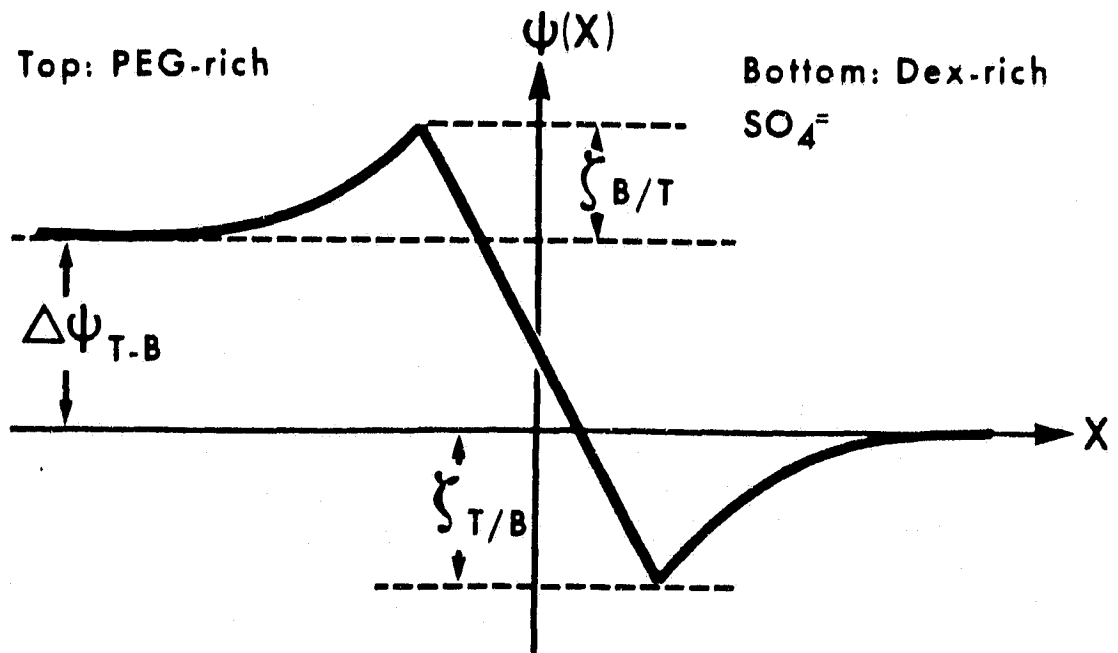


Figure 7B



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STUDIES ON THE INTERFACIAL TENSION OF DEXTRAN/PEG SYSTEMS.

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The interfacial tension,  $\gamma$ , of the two phase system consisting of Dextran T40 and PEG 6000 was measured as a function of tie line length on the phase diagram by means of the rotating drop technique. The dependence of  $\log \gamma$  on tie line lengths is nonlinear. For shorter tie line lengths Ryden and Albertsson (J. Colloid Interface Sci. 37:219, 1971) reported a linear relationship between  $\log \gamma$  and tie line length, although at tie line lengths comparable to those reported in this study the relationship also appears to become nonlinear.

The effects of including salt in the systems were also examined. When sodium phosphate (0.11 M, pH 7.5) was present the interfacial tension was  $\sim 1.5$  times greater than that for the salt free system of the same tie line length. The added salt also produced a shift in the critical point to lower dextran and PEG concentrations, similar to the effect of lowering temperature. The effect of NaCl (0.15 M) on the interfacial tension was similar too but less marked than the sodium phosphate (0.11 M). The binodial of the system containing 0.15 M NaCl lies between the salt free and the sodium phosphate system. Thus it is apparent that the addition of salt to the phase systems significantly increases the interfacial free energy of the phase systems, an effect that by itself would increase adsorption of cells and particulate matter in the interface.

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ELECTROSTATIC POTENTIALS IN TWO POLYMER AQUEOUS PHASE SYSTEMS. S. Bamberger, G.V.F. Seaman and D.E. Brooks, Department of Neurology, Oregon Health Sciences University, Portland OR 97201 and Departments of Pathology and Chemistry, University of British Columbia, Vancouver, Canada V6T 1W5

Two polymer phase systems have proven useful as partition media for cells, the partition coefficient correlating with cell surface charge in dextran/poly(ethylene glycol) (PEG) systems containing divalent an ions. The partition coefficients of  $K_2SO_4$  and KCl were measured and correlated with the Donnan potential difference between the phases, estimated using salt bridges to reversible electrodes. The preference of sulfate for the bottom, dextran-rich phase was shown by equilibrium dialysis experiments to be due to the greater exclusion of the anion by PEG than by dextran. Electrophoretic studies of droplets of one phase suspended in the other revealed relatively large drop mobilities which increased linearly with drop radius and supralinearly with sulfate concentration. The sign of the zeta potential on drops of either phase was opposite to that expected from the Donnan potential, however. The simplest potential profile which is consistent with these observations is one in which a dipole potential is present at the phase boundary oriented so as to reverse the potential gradient locally.

CELL PARTITIONING IN AQUEOUS POLYMER TWO PHASE SYSTEMS AND CELL SURFACE FREE ENERGIES RELATED THROUGH THREE PHASE CONTACT ANGLES. K.A. SHARP, E.A. EVANS, D.E. BROOKS, DEPTS. OF PATHOLOGY AND CHEMISTRY, UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, CANADA, V6T 1W5.

Aqueous polymer two phase systems have been widely used to separate cells on the basis of surface properties by partition between the two phase interface and the top phase, where the partition coefficient  $K = (\# \text{ in TOP} / \# \text{ at INTERFACE})$ . Boltzmann distribution calculations predict that for particles  $> 0.3 \mu\text{m. dia.}$ ,  $K=0$ , since the free energy of adsorption to the interface,  $\Delta G$ ,  $\gg kT$ , where  $\Delta G$  is determined by the liquid-liquid interfacial tension ( $\gamma_i$ ) and the difference in the cell surface free energies in each phase ( $\Delta\gamma$ ).  $\gamma_i$ , the three phase contact angle  $\theta$ , (where  $\cos\theta = \Delta\gamma/\gamma_i$ ) and hence  $\Delta\gamma$  were measured in a system where  $K$  for human RBC's could be increased from 0.02 to 10 by the addition of  $< 1 \mu\text{M}$  PEG-palmitate ester. The partition process could be still modelled by  $K = \exp(\Delta G/kT)$  with an effective temperature of  $5 \times 10^6 \text{ }^\circ\text{K}$ . We hypothesize that the much larger energies associated with fluid shear stresses generated during coalescence and settling of the phases, rather than thermal energies, are responsible for partitioning large particles such as cells.

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ELECTRICAL POTENTIALS ACROSS THE INTERPHASE IN TWO POLYMER AQUEOUS PHASE SYSTEMS. S. Bamberger, G.V.F. Seaman, K.A. Sharp, and D.E. Brooks, Department of Neurology, Oregon Health Sciences University, Portland OR 97201 and Departments of Pathology and Chemistry, University of British Columbia, Vancouver, Canada V6T 1W5.

The partition of cells in dextran/poly(ethylene glycol) (PEG) aqueous phase systems containing divalent anions correlates with the cell surface charge. The Donnan potential difference between the phases in the presence of potassium sulfate was measured by using salt bridges to reversible electrodes. The potential difference remains constant at salt concentrations between 0.003 and 0.1 M, but increases with increasing salt concentration between 0.1 and 0.4 M. The measured potentials agreed well with the potentials calculated from the partition of the salts between the phases. The changes in the potential difference at higher salt concentrations result from deviations in the tie line length. The preference of sulfate for the bottom, dextran-rich phase was shown to be due to the repulsion of the anion by PEG whereas the presence of dextran had no influence on the sulfate partitioning. Electrophoretic studies of droplets of one phase suspended in the other revealed relatively large drop mobilities which increased linearly with drop radius and supralinearly with sulfate concentration. The sign of the zeta potential on drops of either phase was opposite to that expected from the Donnan potential, however. The simplest potential profile which is consistent with these observations is one in which a dipole potential is present at the phase boundary oriented so as to reverse the potential gradient locally.

GRAVITATIONAL LIMITATIONS ON CELL PARTITION SEPARATIONS.

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A thermodynamic analysis of the equilibrium distribution expected for spherical cells partitioning between one bulk phase and the interface of a phase system has been carried out and tested experimentally. The dependence of the partition coefficients of bacteria and cells on the interfacial tension, electrostatic potential between the phases and the binding of PEG ligands obeys the predictions over a range of conditions except that temperature orders of magnitude too high must be assumed. There appears to be a randomizing influence present during the partition process, therefore, which is equivalent in effect to large thermal fluctuations. The most likely sources for this randomizing energy appear to be the mechanical and shear forces acting on cells adsorbed at the phase boundary as the phases settle, tending to pull cells from the interface. If these randomizing effects could be reduced, separations of much higher resolution would be possible because the partition coefficient would be determined only by the thermodynamics of adsorption at the phase boundary, a process which is essentially non-stochastic and which can be controlled. In a reduced gravity environment phase separation would be much slower and the fluid mechanical forces acting on cells lower. An experiment has been proposed to NASA to be carried out on the space shuttle to test these ideas.

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CELL PARTITION AND SURFACE FREE ENERGIES RELATED THROUGH  
CONTACT ANGLES IN TWO PHASE POLYMER SYSTEMS

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The free energy of adsorption of a particle to the interface between two phases ( $\Delta G$ ) is determined by the liquid/liquid interfacial tension ( $\gamma_i$ ) and the difference in the particle surface free energy in each phase ( $\Delta\gamma$ ).  $\Delta\gamma$  and  $\gamma_i$  are related to the contact angle the interface makes with the surface ( $\theta$ ) by Young's equation. Boltzmann distribution calculations predict that for particles  $> 0.3 \mu\text{m}$  dia. the partition coefficient  $K=0$ , since  $\Delta G \gg kT$ . A model system was studied in which  $K$  for human erythrocytes could be increased from 0.02 to 10 by adding  $< 1 \mu\text{M}$  PEG- palmitate ester. The ester/cell surface binding energy in the top and bottom phases, and the energy of ester transfer between the phases were measured as -16.6, -17.9 and 1.38 kT/molecule, respectively.  $\Delta\gamma$  and hence  $\Delta G$  were calculated from measurements of  $\theta$  and  $\gamma_i$  as a function of ester concentration.  $\Delta\gamma$  was only increased by 0.05 kT/bound ester molecule. A thermodynamic model for the effect of an affinity ligand on  $\Delta G$  for a particle is proposed to account for this. This data and the reproducible but statistical nature of cell partition indicate that the process can still be modelled by:  $\ln K = C - \Delta G/kT$ , but with an effective temperature of  $5 \times 10^6 \text{K}$ . We hypothesize that the much larger energies associated with fluid shear stresses generated during coalescence and settling of the phases, rather than thermal energies, are responsible for partitioning large particles such as cells. We thank MRC Canada for support.